SCIENTIFIC VALIDATION OF *"SOODHAGA MEZHUGU''* FOR OVULATION INDUCING ACTIVITY, ESTIMATION OF HORMONE LEVEL AND ANTI-OXIDANT ACTIVITY IN FEMALE WISTAR ALBINO RATS

The dissertation Submitted by

Dr. L. KAVINILAVU

Reg. No: 321612105

Under the Guidance of

Dr. M.D. SARAVANA DEVI M.D (S).,

Dissertation submitted to

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

CHENNAI -106

OCTOBER 2019

GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**Scientific validation of *Soodhaga Mezhugu* for Ovulation Inducing Activity, Estimation of Hormone level and Anti-oxidant Activity in Female Wistar Albino Rats**" is a bonafide and genuine research work carried out by me under the guidance of **Dr.M.D.Saravana Devi M.D(s),** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate,**

Place: Chennai **Dr. L. Kavinilavu.**

GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106.

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Scientific validation of *Soodhaga Mezhugu* for Ovulation Inducing Activity, Estimation of Hormone level and Anti-oxidant Activity in Female Wistar Albino Rats**" is submitted to the Tamilnadu Dr. M. G. R. Medical University in partial fulfillment of the requirements for the award of degree of M.D(Siddha) is the bonafide and genuine research work done by **Dr. L. Kavinilavu** under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Seal & Signature of the Guide**

Place: Chennai

GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106.

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific validation of *Soodhaga Mezhugu* for Ovulation inducing activity, Estimation of hormone level and Antioxidant activity in female wistar albino rats"** is a bonafide work carried out by **Dr. L. Kavinilavu** under the guidance of **Dr. M. D. Saravana Devi M.D(s).,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

Seal & Signature of the HOD **Seal & Signature of the Principal**

Date: **Date:**

Place: Chennai **Place:** Chennai

ACKNOWLEDGEMENT

I would like to acknowledge and extend my cordial credit to the following persons who have made the completion of this dissertation study fruitful.

I hereby pledge my sincere devotion and respect to all the *Siddhars* who guided me eternally and dynamically.

I express my sincere thanks to our **Principal and Heat of the Department**, **PG *Gunapadam*, Prof**.**Dr.R.Meenakumari M.D(S)**, Govt. Siddha Medical College, Chennai for her permission to perform this study and also for her valuable ideas and support throughout the course of the study.

I take this opportunity to express my profound gratitude and deep regards to my guide **Prof. Dr. M.D. Saravana Devi M.D(S), Dept of PG *Gunapadam*** for her exemplary guidance, monitoring and constant encouragement throughout the course of this dissertation. The blessing, help and guidance given by her time to time shall carry me a long way in the journey of life on which I am about to embark.

I would like to express my special gratitude to **Dr. R. Karolin Daisy Rani M.D(S), Lecturer, PG *Gunapadam,*** Govt. Siddha Medical College, Chennai, for his valuable guidance, suggestions for completion of my whole study.

I owe my special thanks and sincere gratitude to my advisor **Dr.V.Velpandian M.D(S)., Ph.D.,** for his support towards my dissertation topic discussions and selection. His guidance helped me in all time of my research work.

I express sincere thanks to **Former Principal and presently Director General Prof. Dr. K. Kanakavalli M.D (S)., Central Council For Research In Siddha, Ministry of AYUSH, Chennai, Govt.of India** for her guidance towards this study.

I wish to express my thanks to co-guide **Dr. K.Rajamma Devi Sorubarani M.D(S).**, Asst. Lecturer, Department of PG Gunapadam for his valuable ideas and suggestions to my study.

I acknowledge my thanks to **Dr. A. Ganesan M. D(S)**, **Dr.S.Shankar M. D(S)., Dr.K.Nalina Saraswathi M.D(S).**, for their support and guidance.

I cordially register thanks to **Dr. Muralidaran Ph.D**., C.L Baid Metha College of Pharmacy, Assistant Professor advanced Centre for research for helping in the pharmacological study and advanced research for his assistance in the toxicity studies.

I extended my gratitude to the animal **Ethical Committee Members** for their approval to do animal studies in pre-clinical studies.

I wish to express my profound gratitude to **Dr. R. Rajesh M.Phil, Ph.D.,** Director, Biogenic research center, Trivandrum, for his valuable work in Antioxidant activity.

I would express my thanks to **Dr. Murugesan, M.Sc,** IIT Anna University who helped in the instrumental analysis of this study.

I acknowledge my thanks to **Mr. Selvaraj M.Sc, M.Phil,** HOD, Bio-Chemistry Department for helping me to prepare the test sample for biochemical analysis of the trial drug.

I would like to acknowledge **Dr.N.Kabilan MD(s),Ph.D, The TamilNadu Dr.M.G.R Medical University** for doing my physicochemical analysis.

I express my thanks to our Librarian **Mr.V.Dhandayuthapani, B.Com, M.Lib.Sc** and staffs for their kind co-operation for my study.

I am also thankful to **Mrs.H.M. Sudha Merlin, D.Pharm,** Pharmacist, Post Graduate Department of *Gunapadam* for her kind co-operation in purification and preparation of the trial drug for my study and successful completion of dissertation.

I would like to thank **Vice Chancellor, The Tamilnadu Dr. M.G.R Medical University** for giving permission to carry out my dissertation work and to the Additional Chief Secretary and Commissioner of Indian Medicine and Homeopathy Department, Arumbakkam, Chennai-106, for giving consent to do the dissertation.

I would like to express my pleasured thankfulness to all my College Staffs, **all my classmates** and intimate friends of PG *Gunapadam* dept for boosting up and comforting me in completion of the study.

Although I wish to thank extends beyond the limits of this format, I would like to thank my friends, and well-wishers for their support and inspiration throughout the dissertation work.

And also, I would like to thank my friends **Dr. S. Sabarmathi, Dr. R. Tamilselvan, Dr. S. Poonkuzhali, Dr. S. Sushmitha, Dr. S. Shakila, Dr. S. Abinaya, Dr. G. Arthi** who lender their hands during my dissertation work.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF CHARTS

ABBREVIATIONS

WHO	**World Health Organisation**
LH	**Luteinizing Hormone**
FSH	**Follicle-Stimulating Hormone**
AMH	**Anti-Mullerian Hormone**
MIH	**Mullerian Inhibiting Hormone**
DM1	**Type 1 Diabetes Mellitus**
HPO A	**Hypothalamic-Pituitary-Ovarian Axis**
ACOG	**American College of Obstetricians and Gynaecologists**
SOGC	**Society of Obstetricians and Gynaecologists of Canada**
OHS	**Ovarian Hyperstimulation Syndrome**
PCOS	**Polycystic Ovarian Syndrome**
SM	**Soodhaga Mezhugu**
CNS	**Central Nervous System**
NIH	**National Institutes of Health**
SHBG	**Sex-Hormone Binding Globulin**
GnRH	**Gonadotrophin Releasing Hormone**
IGFBP-1	**Insulin-like Growth Factor Binding Protein-1**
IGF-1	**Insulin-like Growth Factor-1**
FMR1	**Fragile X Mental Retardation 1**
HDL-C	**High-Density Lipoprotein Cholesterol**
LDLC	**Low-Density Lipoprotein Cholesterol**
FFA	**Free Fatty Acid**
IRS-1	**Insulin Receptor Substrate-1**
DHEA-S	**Dehydroepiandrosterone-Sulphate**
GTT	**Glucose Tolerance Test**
SHBG	**Sex Hormone Binding Globulin**
IE	**Inadvertent enterotomy**
IVF	**In Vitro Fertilization**

POMC	**Pro-opiomelanocortin**
IAEC	**Institution Animal Ethics Committee**
CPCSEA	**Control and Supervision of Experiments on Animals**
PNA	**Prenatal Androgen**
PPA	**Pre-Pubertal Androgen**
DNA	**Deoxyribonucleic**
MIC	**Minimum Inhibitory Concentration**
HPTLC	**High Performance Thin Layer Chromatography**
FT-IR	**Fourier Transform Infra-red Spectroscopy**
ICP-OES	**Inductively Coupled Plasma Optic Emission Spectrometry**
SEM	**Scanning Electron Microscope**
XRD	**X- ray Powder Diffraction**
OECD	**Organization for Economic Co-operation and Development**
CMC	**Carboxyl Methyl Cellulose**
EDTA	**Ethylene-Diamine-Tetra acetic Acid**
RBC	**Red Blood Corpuscles**
WBC	**White Blood Corpuscles**
PCV	**Packed Cell Volume**
GOT	**Glutamate Oxaloacetate Transaminase**
AST	**Aspartate aminotransferase**
GPT	**Glutamate Pyruvate Transaminase**
ALT	**Alanine aminotransferase**
ANOVA	**Analysis of Variance**
ELISA	**Enzyme Linked Immuno Sorbent Assay**
PMS	**Post-Menopausal Syndrome**

GMP	**Good Manufacturing Practices**
LOD	**Loss on Drying**
AGE	**Advanced Glycation End-product**
GSH	**Glutathione**

1. INTRODUCTION

தற்காத்துத் தற்கொண்டான் பேணித் தகைசான்ற
சொற்காத்துச் சோர்விலாள் பெண்.

- திருக்குறள் -அதிகாரம் 6-56[1].

"We have a vision where women and girls live in dignity,

Are healthy, have choices and equal opportunities"

- Foundation for Women Health Research and Development [2].

Health is state of complete physical mental and social well-being and not merely the absence of disease and infirmity. The WHO defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". The health status of women includes their mental and social condition as affected by prevailing norms and attitudes of society in addition to their biological and physiological problems [3].

"Healthy women healthy world"

Women health is an example of population health, the health of a specific defined population. It has been described as "a patchwork quilt with gaps" [4].

World will prosper in knowledge and intellect,

If both men and women are deemed equal.

- ***Subramanya Bharathi.***

Women experience of health and disease differ from those of men, due to unique biological, social and behavioural conditions. Biological differences vary all the way from phenotype to the cellular, and manifest unique risks for the development of ill health [5].

While both men and women contact various conditions, some health issues affect women differently and more commonly. Women bear exclusive health concerns, such as,

- Breast cancer
- Cervical cancer
- Menopause
- Pregnancy
- Heart attack
- Depression and anxiety
- Urinary tract infections
- Sexually transmitted diseases
- Osteoporosis
- Thyroid problems
- Systemic lupus erythematosus [6].

Many women's health conditions go undiagnosed and most drug trials do not include female test subjects.

Hormone plays vital role in women health. Nowadays Women facing the main problem is hormone imbalance. Hormone distrubances are increased with stress and then result in Polycystic ovarian disease. Work pressures lead them to eat more of junk food which leads to obesity and other health related issues [7].

Polycystic ovarian disease or syndrome was earlier known as Stein-Leventhal syndrome. 1% female population suffers from PCOS, and the patients are mostly 15 to 25 years of age. PCOS includes chronic non-ovulation and hyperandrogenaemia associated with normal or raised oestrogen (E_2), Altered LH/FSH ratio (raised LH and low FSH ratio) [8].

PCOS is a heterogeneous endocrine disorder, leading to several health complications, including menstrual dysfunction, infertility, hirsutism, acne, obesity and metabolic syndrome [9].

Anti-Mullerian hormone (AMH), also known as Mullerian inhibiting hormone (MIH), is a glycoprotein hormone structurally related to inhibin and activin from the

transforming growth factor beta superfamily, whose key rules are in growth differentiation and folliculogenesis [10].

Anti-Mullerian hormone (AMH) levels are increased in polycystic ovarian syndrome (PCOS), but it is not known whether other forms of hyperandrogenism, such as PCOS observed in women with type 1 Diabetes Mellitus (DM1), are also associated with elevated AMH levels.

Causes: Causes of PCOS is unknown. But Doctor's believe that high levels of male hormones prevent the ovaries from producing hormones and making eggs normally. Genes, insulin resistance, and inflammation have all been linked to excess androgen production [11].

Prevalence: PCOS is a common female endocrine disorder with prevalence ranging from 2.2% to 26%. Most reports have studied adult women with age ranged from 18 to 45 years. The aim of this study was to find the prevalence of in Indian adolescents. In Indian adolescents is 9.3% [12].

Signs & symptoms:

- Irregular periods
- Poly cystic ovaries
- Hirsutism
- Obesity
- Acne
- Alopecia
- Skin tags
- Acanthosis nigricans
- Infertility [13]

Pathophysiology: The endocrinologic abnormality of PCOS begins soon after menarche. Chronically elevated luteinizing hormone (LH) and insulin resistance are 2 of the most common endocrine aberrations seen in PCOS. The genetic cause of high LH is not known. It is interesting to note that neither an elevation in LH nor insulin resistance alone is enough to explain the pathogenesis of PCOS.

Obesity, which is seen in 50% to 65% of PCOS patients, may increase the insulin resistance and hyperinsulinemia. One important caveat is that the correlation

between hyperandrogenism and insulin resistance has been recognized in both obese and nonobese anovulatory women. Thus, it is important to realize that a nonobese patient may also have insulin resistance.

Regular ovulatory cycles at puberty depends on a complex series of interactions involving the hypothalamus, anterior pituitary and ovary (HPO axis) in Polycystic ovarian disease normal physiological function are distrubed because of defect in hypothalamo pituitary and ovarian axis leads to hormonal imbalance[14].

Complications: Women with PCOS are more likely to develop certain serious health problems. These include type 2 diabetes, high blood pressure, problems with the heart and blood vessels and uterine cancer. Women with PCOS often have problems with their ability to get pregnant (fertility) [15].

The American College of Obstetricians and Gynaecologists (ACOG) and Society of Obstetricians and Gynaecologists of Canada (SOGC) indicate that lifestyle modifications such as weight loss and increased exercise in conjunction with a change in diet consistently reduce the risk of diabetes [16].

Treatment: Typical ways to treat hyperandrogenism include cosmetic procedures (specifically for hirsutism), estro-progestins, antiandrogens alone or combined with ethinylestradiol and if necessary, insulin sensitizers, particularly metformin. Antiandrogens may represent an alternative way to improve both hyperandrogenaemia and hirsutism [17].

Complications of treatment:

- Multiple pregnancy
- Ovarian Hyperstimulation Syndrome (OHS)
- Adhesion formation in surgical procedure[18].

Siddha system of medicine is enriched with unique and peculiar aspects with treasure house of secret science. The meaning of "*Siddh*" is endless knowledge, hence Siddha denotes unlimited knowledge. Another possible explanation is that those who achieved perfection are called *Siddhars*. Since the word *Siddhi* means achievement of perfection. The system of medicine revitalized and rejuvenates the internal organs and provide guidelines for healthy lifestyle for healthy living.

The *Siddhars* uniformly believed that a human being is composed of 96 basic factors. These 96 factors are which they called as basic "*Thathuvas*", these *Thathuvas* are universal to all human beings.

They classified various female diseases and had amazing solutions for its curative measures and also handled with preventive aspects.

In *Siddha* system of medicine, numerous single herbals, poly herbal formulation is available to treat PCOS. Such as,

- *Moosambara mezhugu,*
- *Lavana chendhooram,*
- *Sanga thiravagam,*
- *Kariyuppu parpam,*
- *Attathi chooranam,*
- *Saampiranippoo kuzhigai.*

So, I hope that this *Siddha* system of medicine will cure the PCOS, that's why I prefer to choose the medicine ***SOODHAGA MEZHUGU***. One of the main herbals & mineral formulation mentioned for PCOS is **"*Soodhaga Mezhugu*"** which contains *Vengarathool [Sodium biborate], Valendhrapolam [Commiphora myrrha], Kunguma poo [Crocus sativus], Kirambu thylum [Syzygium aromaticum] by* Ovulation inducing activity, Estimation of hormone level, Anti-oxidant activity in animal model.

Hence, this ***SOODHAGA MEZHUGU*** from our treasure of *Siddha* system of medicines would provide the society with a drug that is cost effective, Safe, Promising drug for PCOS.

2. AIM AND OBJECTIVES

AIM:

The present study will be focused to validate the **PCOS** curing efficacy of "***SOODHAGA MEZHUGU"*** by Ovulation Inducing Activity, Estimation of Hormone Level, Anti-oxidant Activity in animal model.

OBJECTIVES:

- Collection of relevant literature from *Siddha* text book at the same time compared and collected with modern aspect for present study.
- Systematic identification of drug material through microscopic and macroscopic method.
- Detoxification and manufacture of drug material according to the *Siddha* classical literature.
- Standardization of trial drug through the following steps:
    - Macroscopic Methods
    - Microscopic Methods
    - Physical Methods
    - Chemical Methods
    - Biological Methods
- To analyze physicochemical property of the drug.
- Identifying the presence of acid and basic radicles through biochemical analysis.
- Identify the chemical structure of the drug through the Instrumental analysis.
- To determine the toxic effect of trial drug with the help of OECD guideline (Acute 423 and Repeated Oral Toxicity 407).
- Pharmacological activity of the trial drug ***Soodhaga Mezhugu (SM)*** is proved by the following method Ovulation Inducing Activity (Estrodial induced PCOS in Female Wistar albino rats), Estimation of Hormone Level (Estrodial induced PCOS in Female Wistar albino rats), Anti-oxidant Activity (DPPH assay method).

3. REVIEW OF LITERATURE

3.1. DRUG REVIEW

3.1.1. GUNAPADAM ASPECT

The following drugs are the ingredients of *Soodhaga mezhugu* (SM)

- *Vengarathool [Sodium biborate]*
- *Valendhrapolam [Commiphora myrrha]*
- *Kunguma poo [Crocus sativus]*
- *Kirambu thylum [Syzygium aromaticum]*

1.Vengarathool (Sodium biborate)

Chemical name: *Sodium biborate*

Other name of *Vengarathool:*

- *Porikaram*
- *Karam*
- *Urukkinam*
- *Urukkumithran*
- *Danganam*
- *Thoomathaiadakki*

The Borax is roasted in a pan till the moisture completely evaporates. By this method of purification, the borax became in purified form and used in the preparation of medicine.

It is one among the type of salt (*Kaarasaaram*). It comes under synthetic salt (*Saeiyarkai uppu*). Borax is obtained in California abundantly. It is also found in Tibet and Nepal. Naturally it is obtained along with sand and dust.

Properties of Borax:

- Color is white, clear, shining with some angles
- Soluble in water
- Insoluble in alcohol
- Taste is sweet with astringent

The Borax available in the shops are not pure. Hence four parts of hot water and a small amount of Calcium carbonate(lime) are added to it, filtered, insulated and heated till the water evaporates completely. The salt so obtained is pure and can be used.

If borax expands to air, white powder is deposited on the surface. It is very important drug for preparing Parpam, Chendhooram and Guru-Kuligai.

Taste : Sweet, Astringent.

Potency : Heat

Action

1. **Internally:**
    - ❖ Putrefacient
    - ❖ Diuretic
    - ❖ Emmenagogue
    - ❖ Refrigerant
    - ❖ Lithotripsic
2. **Externally**:
    - ❖ Astringent
    - ❖ Antiseptic
    - ❖ Alternative
    - ❖ Neutralizer

General character:

"காரமென்று இதற்குப்பேர் வந்தது எது
கட்டுமே அறுபத்து நாலு தாதும்
காரமென்று இதற்குப்பேர் வந்த தாலே
கடிசான உபரசநூற் றிரண்டும் சத்தாம்
காரமென்று இதற்குப்பேர் வந்ததென்றால்
கட்டாத சாரந்தான் இதற்குள் கட்டும்
காரமென்று இதற்குப்பேர் வந்த தாலே
களங்குகுரு சிந்தூரத்து ஆதி காணே".

-போகர் ஏழாயிரம் இரண்டாம் காண்டம்.

Special characters of Vengaram

"நெளியான உருக்கினத்தைச் சொல்லக் கேளு
நேரான வெண்காரம் ஜவ்வாதுவீர
மொளியான ரெங்கிட்டால் உருக்கினத்துக்காத"

- சட்டமுனி வாத காவியம்.

- It makes 64 *paadanam* as *Kattu*
- It makes 120 *ubarasam* as *sathu*
- It is important *(aathi*) for preparing *Kalangu, Chendooram* and *Guru.*

General characters:

"சொறிபுடையெண் குன்மநமை சோரி யாசம்
பறிகிரகணி கல்லுரனம் பன்னோய்-நெறியைத்
தடங்கணங்க பங்கிருமி சர்ப்பவிடஞ் சந்நி
யிடங்கணங்க லக்கிற்போ மெண்."

- குணபாடம் தாது சீவம் வகுப்பு.

Indications:

- It cures Toad skin, Itching, Venereal ulcer with pus and Ulcers on the nipple.
- It is also used to cure Gastric ulcer, Hemorrhoids, Stomatitis, Itching, Indigestion.
- Borax is used to cure Amenorrhea, Menorrhagia, Dysmenorrhea, Delayed labor.
- Borax is used to cure Hemiplegia and Urinary Tract Infections.

Purifications:

- Borax is bundled, kept buried in buffalo's dung for 3 days, then washed and dried.
- Borax is soaked in buffalo's urine for 72 minutes.
- Fried in an earthen pan and triturated with lemon juice or vinegar and dried.
- Roasted in a pan till the moisture completely evaporates.

Uses:

- Borax is mixed with water. A cotton cloth is dipped and it's applied externally for wounds.
- Borax with pig ghee applied over the painful anus for anorectal diseases.
- Borax with tender coconut cures urinary tract infection.
- It is mixed with water and used for cleaning the eyes in eye diseases.
- Borax with betel leaf prevents fever with rigor [19].

Other Preparations from borax:

- *Venkaraparpam*
- *Venkaramathirai*
- *Venkarakattu*
- *Vengara mathu*: Roasted borax with honey cures glossitis.
- *Venkaraneer*

Chooranam

- Deepakini chooranam,

Maathirai

- *Aanantha pairavam*
- *Emathanda kuligai*
- *Venkaramathirai*

Chenthooram

- *Mandoorachendhooram*

Kuzhambu

- *Agasthiyar kuzhambu*
- *Kumatti kuzhambu*
- *Gowsigar kuzhambu*

Mezhugu

- *Gunmakudori mezhugu*

2. Valendhrapolam (Commiphora myrrha)

Botanical Name: *Commiphora myrrha*

Other names

- *Kunkumadeepam*
- *Kundhru*
- *Vellaippolam*
- *Vellaathirapolam*
- *Meeru*

Family - Burseraceae

Vernacular names:

Eng - Myrrh

Hin - Bol, Hirable

Kan - Bola

Mal - Narumpasamaram, Narumpasa

San - Bolah, Rasagandhah

Tam - *Vellaippolam*

Tel - Balimtra – Polam

Habitat:

- A small tree which is the source of Herabol myrrh.
- The gum resin exudate from wounds in the stem is pale yellow at first and later solidifies to brown-black.

Part used - Gum

Taste - Bitter

Potency - Heat

Division - Pungent

Action:

- Stimulant
- Expectorant
- Emmenagogue
- Stomachic
- Carminative

General characters:

சூலைகய ரோகம் சொறிகரப்பான் குன்ம மிவை
ஆலைவா யிங்கோல்போல் ஆகுங்காண் – நீலசக்கர
வாளமெனத் தோற்றுமுலை மாதரசே! வாலேந்திர
போளந் தனையெடுக்கும் போது.

- *அகத்தியர் குணபாடம்.*

Indications : Acute pain, Tuberculosis, Pruritis, Eczema, Peptic ulcer[20a].

Preparation of Myrrh:

- *Moosambara mezhugu.*

3.Kunkumapoo (Crocus sativus)

Other name:

- *Kashmiram*
- *Gnyazhal poo*

Vernacular name:

Sanskrit - *Bhavarakta*, *Kumkuma*.

English - Saffron

Hindi - *Kumkum, kesar*

Telugu - *Kumkuma-puvvu*

Malayalam - *Kumkumada puvvu.*

Habitat:

An autumnal dwarf herb, a native of Levant in Asia Minor, now cultivated on a small scale in Kashmir and around Quetta.

Parts used:

Dried stigmas and tops of the styles of Crocus sativus, which constitute the saffron of commerce compressed into cakes and called "cake saffron" the ordinary saffron being called Hay saffron.

Properties and uses:

Taste : Bitter

Potency : Heat

Division : Pungent

Action

- Stimulant
- Stomachic
- Antispasmodic
- Emmenagogue

General characters:

"விந்துநட்டந் தாகமண்டம் மேகசலஞ் சூலைகபம்
உந்துசுரம் பித்தங்கால் உச்சிவலி முந்துகண்ணில்
தங்குமப்பூ வோடுறுநோய் சர்த்தியவை நீங்கவென்றால்
குங்குமப்பூ ஓரிதழைக் கொள்."

- *அகத்தியர் குணபாடம்.*

Indications:

It cures Reduced Sperm Count, Thirst, Diabetes, Neuritis, Kabhasuram, Headache, Cataract, Eye related diseases, Vomiting, Ear related diseases, Sinusitis, Taste of sweetness in mouth. It also Expels Lochia.

Therapeutic Uses:

- It is used generally as a condiment for its aromatic odor and beautiful coloring matter.
- Medicinally, it is used in small doses, in fevers, melancholia, enlargement of liver and in spasmodic cough and Asthma and in catarrhal affections of children.
- It is given in Anemia, Chlorosis and Seminal debility.
- As a stimulant and Aphrodisiac, it is considered to be a sovereign remedy, not to be excelled in virtue by the whole range of drugs in the Materia medica.
- It gives the urine a yellow color. It is given in Rheumatism and Neuralgia and to children with ghee in looseness of the bowels.
- It is given also to relieve flatulent colic, Amenorrhea, Dysmenorrhea, Leucorrhoea etc.
- Pessaries of saffron are used in painful Infections of the uterus.
- Externally saffron is used in head ache in the form of paste, also applied to bruises and superficial sores.
- It is an excellent palliative for Hemorrhoids.
- Saffron is used in snake bite also[20b].

Preparation of Saffron:

Maathirai

- *Korosanai maathirai*
- *Maha elathi kuligai*
- *Kunguma poo maathirai*

Karuppu

- *Kasthoori karuppu*

Mai

- *Nandhimai*

4. Kirambu (Syzygium aromaticum)

Other names :

- *Lavangam*
- *Anjukam*
- *Tirili*
- *Varangam*
- *Chosam*

Vernacular names

Tamil	:	*Kirambu*
English	:	Cloves, clove tree
Sanskrit	:	*Lavangam*
Telugu	:	*Lavangalu*
Kannadam	:	*Lavanga*
Hindi	:	*Long*
Malayalam	:	*Karampu*

Part used:

Dried flower buds, fruit and oil.

Properties and uses:

Taste	:	Pungent
Potency	:	Heat
Division	:	Pungent

Action

- Carminative
- Antispasmodic
- Appetizer

Habitat:

Clove is a dense and handsome evergreen tree that can grow around 20 meters tall. The short bole can be around 25cm in diameter.

The plant has been valued as a spice for at least 2,500 years. It is commonly cultivated in the tropics, especially southeaster Asia but also Africa and the Americas, for its dried, unopened flowers and the essential oil derived from them.

General characters:

"பித்தமயக்கம் பேதியொடு வாந்தியும் போம்
சுத்தவிரத்தக் கடுப்புந் தோன்றுமோ-மெத்த
இலவங்கங் கொண்ட வருக்கேற் சுகமாகும்
மலமங்கே கட்டுமென வாழ்த்து."
"சுக்கிலநட்டங் கர்ணசூர்வியங் கலாஞ் சனந்தாட்
சிக்கல் விடாச்சர் வாசியப்பிணியு- மக்கிக்குட்
டங்கப்பூ வோடு தரிபடருந் தோன்றிலில்
வங்கப்பூ வோடு ரைத்துவா"

\- *அகத்தியர் குணபாடம்.*

Indications: It cures Giddiness, Diarrhoea, Dysentery, Vomiting, Anal fissure and Cataract.

Therapeutic uses

- Relieve Flatulence, Gastric irritability, Dyspepsia and to Increase the flow of saliva
- Given to appease thirst.

Decoction:

It is used for arresting vomiting and curing digestive disorders like indigestion, colic, flatulence, dyspepsia etc.

It is specially recommended for pregnant women in case of vomiting and rumbling noise of the stomach.

The oil distilled from this forms and esteemed remedy for tooth-ache[20c].

Preparation of Clove:

Kirambu thylum

Maathirai:

- *Kunkumapoo maathirai*
- *Kapada mathirai*
- *Amurthathi kuligai*
- *Asta pairava kuligai*
- *Kalyana vairavam*

Thylum:

- *Kirambu thylum*

3.1.2. MODERN ASPECT OF VENKAARAM:

1. BORAX (வெங்காரம்) [21]:

Other name **:** Sodium tetraborate or Disodium tetraborate.

Chemical Name **:** Sodium biborate

Fig.No.1. Borax (வெங்காரம்)

General properties:

- **Chemical formula** : Hydrated sodium borate $Na_2B_4O_7$ -$10H_2O$
- **Color** **:** White powder with soft colorless crystals.
- **Weight** **:** 201.22 g/mole and
- **Melting point** **:** 741 degrees Celsius.
- **Boiling point** **:** 320 Celsius.
- **Density** **:** 1.73.
- **The pH** **:** alkaline.
- **Solubility** **:** 6g to 100g of water.
- Borax is stable and non-corrosive in the presence of glass.
- **Source:**

On seashore it dried up in lakes of India and Tibet. It is also found in the mud of lakes surrounded by hills of Nepal [(22)].

Chemical properties:

The following are several borates of Sodium

- Sodium tetra borate-$Na_2B_4O_7 10H_2O$ or anhydrous form
- Sodium meta borate-$Na_2BO_4H_2O$
- Sodium per borate -$NaBO_2H_2O_3H_2O$
- Sodium Penta borate-$Na_2B_{10}O_{16}10H_2O$

Commonly Sodium tetra borate was used for all kinds of purposes.

Specific gravity of borax: 1.7

Character:

Borax was composed mainly of boric acid and soda. In the native state borax was impure saline incrustation of a dirty white in colour. It exists as a crystalline tough masses or translucent irregular masses. It was exposed to air becomes opaque in nature. The natural colour of borax was greyish-white.

Purification:

Roasted in a pan till the moisture completely evaporates.

Action:

- ❖ Anti-septic
- ❖ Diuretic
- ❖ Emmenagogue
- ❖ Antacid
- ❖ Astringent and Local sedative

Borax has four special centres of action [23],

- ➢ Mucous membranes - Aphthous inflammation
- ➢ Sexual organs in women - Stimulates menstruation
- ➢ Skin - Slight injuries suppurate
- ➢ Locally - Powerful antiseptic and disinfectant.

Uses of borax:

External

Borax was used for disinfectant and antiseptic and it is much more active in preventing than in inhibiting decomposition.

Internal

- ➢ They are largely used to keep healing the wounds and ulcers. The action was exhibited locally.
- ➢ According to Indian Materia medica the Borax was used internally in dose varying from 10-30 grains, in acidity of stomach, amenorrhea, dysmenorrheal, menorrhagia, puerperal convulsions and to promote uterine pain during labor
- ➢ It was taken internally. It is said to relieve irritability of the bladder.
- ➢ In rare cases its use has caused either psoriasis, a popular eruption especially marked near the elbows, an erythematous rash or eczema. Nausea, loss of appetite, vomiting, and diarrhea may be produced.
- ➢ Borax has been given in CNS disorder epilepsy. As it has an antiseptic it has been given internally in typhoid fever and phthisis, but with less benefit [24].

2. MYRRH (வாலேந்திரபோளம்):

Fig.No.2. Myrrh (வாலேந்திரபோளம்)

Classification:

Kingdom	- Plantae
Division	- Magnoliophyta
Class	- Magnoliopsida
Order	- Sapindales
Family	- Burseraceae
Genus	- *Commiphora*
Species	- *myrrha* [25]

Distribution:

Indigenous to North-East Africa especially Somalia Island. Collected in Saudi Arabia, Abyssinia, Iran, Thailand and sold in Indian bazars[26a].

Description:

The Commiphora species that serve as source of myrrh, are thorny shrubs or small trees that grow to up to 3 meters high. A pale yellow-white viscous liquid exudes

from natural cracks or fissures in the bark or from fissures cut intentionally to harvest the material. When air-dried, these exudates harden into a reddish-brown mass that often contains white patches. These tears are approximately the size of a walnut and from the basis of myrrh resin. Myrrh is usually collected in the summer months (Leung, 1980; Evans, 1989; Michi and Cooper, 1991)

A small tree which is the source of Herbal myrrh.

The gum resin exudate from wounds in the stem is pale yellow at first and later solidifies to brown-black.

Parts used: Gum.

Chemical Constituents:

The major constituents of the essential oil identified from the resin of *Commiphora myrrha* were α-elemene (12.86%), 7-isopropyl-1, 4-dimethyl-2-azulenol (12.22%), curzerene (11.64%) and germacra-1(10)7, 11-trien-15-oic acid,8,12-epoxy-6-hydroxy- c-lactone (6.20%). In both DPPH scavenging and Fe^{2+} chelating assays [(27)].

Myrrha is an oleo-gum-resin obtained from the stem of C. molmol (Evans, 1989; Michi and cooper, 1991). It contains 2% to 10% of a volatile oil composed predominantly of sesquiterpenes, sterols, and steroids. Water soluble gum portion (30%- 60%) contains polysaccharides and proteins as well as ethanol-soluble resins (25%-40%). After undergoing hydrolysis, the gum produces a variety of sugars. 9F anesthetic, antibacterial, antifungal, and hypoglycemic effect (Hanus et al, 2005; 8 Zhu et al, 2003). When oleo-gum-resin is mixed with water it forms an emulsion (El Ashry et al, 2003). 2.2.4. Antimicrobial activity

Action:

Myrrh is thought to mediate its anti-inflammatory activity through inducing haem oxygenase activity. It appears to produce an analgesic effect associated with its suppression of prostaglandin production. Compounds in myrrh bind to and inhibit the farnesoid X receptor resulting in a reduction in low density lipoprotein.

Properties and uses:

The gum is bitter, acrid and astringent, acrid after the process of digestion, thermogenic, digestive, carminative, expectorant, intellect promoting, aphrodisiac, anthelmintic, anti-inflammatory, diuretic, sudorific, ophthalmic, antiseptic, stimulant and tonic, and is useful in vitiated conditions of *Vata*, *Pitta* and *Kapha*, stomatitis, dyspepsia, helminthiasis, amenorrhea, dysmenorrhea and other menstrual disorders, bronchitis, asthma, phthisis, ophthalmia, spongy gum, pharyngodynia, rheumatoid arthritis, sciatica, wounds and ulcers, inflammations, strangury and skin diseases.

The herbal myrrh is also used in perfumery, mouth washes, dentifrices and in religious ceremonies as incense. This is reported to be used by ancients for embalming[(26b)].

Myrrha has been used for centuries as incense (Evans, 1989), and for medicinal purposes (Michi and Cooper, 1991), medicinally, it has been used as an astringent, antiseptic, anti-parasitic, antitussive, emmenagogue, and antispasmodic agent. It was commonly included in mixtures used to treat worm, wounds, sepsis, cough in children, skin and mouth infections, and as suppository form to treat proctitis (Michio and Cooper, 1991). Myrrh has also been reported to treat gout, headache, jaundice, throat ailments indigestion, fatigue, and paralysis (Greene, 1993). It has also been used in variety of 9 infectious diseases, including leprosy and syphilis and to treat cancers (El Ashry et al, 2003) [(27)].

3. SAFFRON (குங்குமப்பூ):

Fig.No.3. Saffron (குங்குமப்பூ)

Classification:

Kingdom	- Plantae
Class	- Monocots
Order	- Asparagales
Family	- Iridaceae
Sub family	- Crocoideae
Genus	- *Crocus*
Species	- *sativus*

Distribution:

The plant is a native of south Europe and is cultivated in Spain, France, Italy, Greece, Turkey, India and China.

Saffron thrives well in cold regions with warm or sub-tropical climate. It requires a rich, well dried sandy or loamy soil. The plant is propagated vegetative by bulbs. Bulbs once established continue to live for 10 to 15 years [28].

Description:

A small bulbous perennial, 15-25 cm high, leaves radical, narrowly linear, channelled, leaf sheaths closely reticulate, flowers blue, scented, appearing with leaves,

throat of perianth bearded, anthers yellow, ovary three-celled, stigmas orange coloured, trifid on filiform style tops, fruits loculicidal capsules.

The saffron of commerce is the dried style tops with orange coloured stigmas.

Parts used:

Dried stigmas and tops of the styles of *Crocus sativus*, which constitute the saffron of commerce compressed into cakes and called "cake saffron" the ordinary saffron being called Hay saffron.

Macroscopic characters:

The dried drug consists of loosely matted mass of dark orange-reddish brown flattened brittle threads of stigma and upper parts of styles each separated piece shows three stigmas attached to a very short or about 1 cm long cylindrical style, the stigma is 2.5 to 3 cm in length, with a long cylindrical tube, narrow at the base getting slowly broader and flattened towards the upper extremities where it slits longitudinally on the inner side, the lips of the tube are irregularly notched and the margin is papillose, surface is longitudinally striated and occasionally studied with pollen grains. Taste is mildly bitter and odour characteristic.

Microscopic characters:

Diagrammatic TS passing through the centre of the flattened stigma is horse-shoe shaped and shows rows of vascular bundles embedded in the parenchymatous mesophyll tissue lying in between the upper and lower epidermis.

Detailed TS shows a layer of upper and lower epidermis and 10 to 15 rows of parenchymatous mesophyll tissue filled with orange red coloured pigments and traversing with conjoint collateral small vascular bundles.

Powder:

Shows transversely cut fragments from the tubular portion of the stigma showing epidermis and mesophyll cells filled with orange coloured pigment and vascular bundle fragments of the papillose epidermis of the stigma from the apical portion in surface view and cut longitudinally large spherical thick-walled pollen grains and their broken fragments, scattered as such throughout transversely cut fragments of the style showing papillose epidermis and cortical cells filled with pigment, longitudinally cut fragments of vascular bundles showing spiral tracheid's and vessels[29].

Chemical Constituents:

Three crystalline colouring matters.

- α – crocetin
- β – Crocetin
- δ – Crocetin

Bitter substances

- Fatty oil - 18-13.4%
- An essential oil - 1.31%
- Moisture - 9.14%
- Nitrogen - 2.22 - 2.43%
- Ash - 5-7%

Chemical Composition:

- Protein - 12.6%
- Fixed oil - 4.7%
- Volatile oil - 0.8%
- N. free extract - 57.3%
- Starch equivalent - 12.0%
- Fibers - 4.9%
- Ash - 4.0%

Saffron contains a mixture of yellow glycosides, crocin being a yellow red pigment, on hydrolysis crocin yield gentiobiose and crocetin.

Average composition of commercial saffron:

- Water - 15.6%
- Starch and Sugar - 13.35%
- Essential oil - 0.6%
- Fixed oil - 5.63%
- Total N. Free extract - 43.64%
- Essential oil - 1.337%
- Fixed oil - 13.4%

The ash is rich in potassium and phosphorous and contains traces of boron.

Saffron contains the glycosides, crocin and picrocrocin together with Lycopene (Beta-carotene), grana carotene and zeaxanthin and a crystalline hydrocarbon.

Crocin is the chief colouring principle in saffron on hydrolysis. It yields the carotenoid pigment crocetin.

The colourless bitter glycoside, Picrocrocin gives on hydrolysis, glucose and the aldehyde safranol.

The essential oil of saffron deposits on standing stearoptene probably a tertiary alcohol [30].

Action:

It has a peculiar aromatic odour and a bitter, pungent taste, it is stimulant, Aphrodisiac and stomachic, slightly anodyne and antispasmodic, it has also Emmenagogue virtues, in over- doses it is narcotic poison. It is used in small doses ¼ to ½ grain, ordinary dose is 1 to 3 grains. "The essential oil from Crocus sativus when passed through Pharmacological tests showed all the characteristic features of an essential oil, therefore its Aphrodisiac virtue are probably due to the slight stimulation of the central nervous system which is common to all essential oils". (Chopra's "I.D of me").

Medicinal uses:

- Saffron is used principally for its coloring and flavoring properties.
- It is used as sedative and Emmenagogue. It is used occasionally in exanthemata's disease to subside eruptions.
- It is used in fevers, Melancholia and enlargement of the liver.
- It is also stimulant and stomachic.
- Saffron is highly valued spice and coloring agent often cited folk remedy for various types of cancer (e.g.) tumors of abdomen, bladder, ear, eye, kidney, spleen, stomach, tonsils. It is used as a nervine sedative and given in fevers melancholia and enlargement of liver.
- In Europe, used as carmine, dysmen and hysteria and Aphrodisiac.
- Unani medicine used as a cardio tonic and strengthens inner part of the body preventing premature ejaculation.
- Used as Blood purifier.

- The stigma of *Crocus sativus* was investigated for its effect on the blood coagulation. Fibrinolysis system and platelet aggregation. It showed remarkable inhibitory effect on blood coagulation.

1. CLOVES (*கிராம்பு*) :

Fig.No.4. Cloves (*கிராம்பு*)

Fig.No.5. Clove oil (*கிராம்பு தைலம்*)

Classification:

Kingdom	: Plantae
Class	: Eudicots
Subclass	: Rosids
Order	: Myrtales
Family	: Myrtaceae
Genus	: *Syzygium*
Species	: *aromaticum*

Distribution:

A tree cultivated in many parts of the world and extent in south India. Tamil Nadu and Kerala.

Description:

An evergreen tree, 9-12 m high or more. Leaves ovate-oblong, acute at both ends, gland-dotted, fragrant, Flower-buds borne in terminal, small clusters of branches, greenish; pink at maturity, aromatic. Fruits fleshy, dark pink drupes. Seeds oblong, grooved in one side.

Flowers between January-June and fruits later.

Parts used: Flower bud

Macroscopical characters

Flower buds are measuring 10 to 17.5mm in length. Colour- Dark brown to black four-sided hypanthium readily exuding oil when pressed odour strongly aromatic taste pungent. Aromatic followed by slight tingling of the tongue

Microscopical characters

Small number of stone cells and prismatic crystals of calcium oxalate present in stalk. Stamens each with an oil gland in the apex of the connective, triangularly ventricular pollen grains. Other walls showing a typical fibrous layer, schizolysigenous glands found in all parts.

Powder

Dark brown; fragments of parenchyma showing large, oval, schizolysigenous oil cavities; spiral tracheid and a few rather thick walled, spindle shaped fibres calcium oxalate crystals in rosette aggregates fragments of anther walls with characteristic reticulated cells, pollen grains numerous [(31)].

Chemical constituents:

Mainly β-caryophyllene eugenol and its acetate, methylsalycylate η-amyl carbinol, benzyl alcohol, dimethyl furfural, furfuryl alcohol, α-methyl furfural, methyl alcohol, methylenate, methyl furfuryl alcohol ethyl-n-hepatylketone, methyl-n-hepatylcarbinol, valeraldehyde, vanillin, 2,6-dimethyl-5-hydeozy-7-methoxychromone (Eugenitin), 2,8-dimethyl-5,7-dihydroxychromone (Isoeugenol), 2-methyl-5-hydroxy-7-methoxychromone (Eugenine), 2,4,6-trimethoxybenzoyl acetone (Eugenone), from flowers-bud oil of wild cloves; furfural, methyl alcohol, naphthalene (Clove stem oil);

benzaldehyde, carvacrol, α-humulene, meth eugenol, eugenine, eugenone, epoxydihydrocaryophylle, methyl-n-amyl ketone, methyl pentanone, naphthalene (Leaf oil) caryophylla-3(12), 6-dien-4-ol, caryophylla-3(12), 7(13)-dien-6 α-al, caryophyllene oxide, 2-hydroxy-4,6-dimethoxy-5-methylaceto-phenone (Clove oil) galactose, glucose, fructose, rhamnose, sucrose, xylose, Gallo tannic and oleanolic acids are isolated from the cloves.

Medicinal Uses

- **Dried flower-buds:** Antispasmodic, aromatic, carminative, stimulant
- Beneficial in colic, dyspepsia, flatulence and various forms of gastric irritability, sore throat and in strengthening of gum, infusion given to allay thirst.
- Paste applied externally with much benefit in coryza and headache.
- Employed in Ayurvedic formulations for bronchitis, debility, dyspepsia and giddiness.
- Clove oil is antiseptic, local anesthetic, counter irritant and rubefacient.
- Externally applied in lumbago, neuralgia, rheumatic pains, sciatica and toothache [32].

3.2. DISEASE REVIEW

3.2.1. SIDDHA ASPECT

SOOTHAGA VAYU

Other names:

- *Soodhaga kattu*
- *Ruthuneer katti*
- *Soodhaga katti*
- *Soodhaga thiratchi*
- *Soodhaga noi*
- *Soodhaga kolaru*

"சித்தான கர்ப்பத்தில் சேர்ந்திடும் இரத்தந்தான்
வத்தாம் வுருண்டு வாயுபோல் ஓடிடும்
வற்ற பசிபோகும் உழன்றே இறைந்திடும்
வற்றாக கழிச்சலாம் வன்சூதக வாயுவே"

- திருமூலர் கருக்கிடை வயித்தியம் 600[33].

Soodhaga vayu refers to a condition of building up of or accumulation of *vayu* and blood in the uterine cavity. This runs around the womb and result in loss of appetite, flatulence and dysentery.

"கேளுமே சூதகத்திலக்கினி வாய்வு
கெடுத்துவிடும் மாதவிடாய் கட்டி போகும்
ஆளுமே கருக்குழியும் தூர்ந்து தேகம்
அப்பனே யுதிரமது அடிமூலத்தில்
நீளுமே சூதகத்தில் வாய்வு தோன்றி
நேரான அடிவயிறு வலிப்புக் காணும்
பாளுமே தலைவலிக்கும் இடுப்பு ளைச்சல்
பக்குவமாய் மருந்துண்ணத் தீருந்தானே"

- ஆவியளிக்கும் அமுதமுறைச் சுருக்கம் [34].

Soodhaga vayu may be compared to Poly cystic ovarian syndrome, which is characterized by *vayu* accumulation in the uterus may lead to amenorrhea, lower abdomen pain, head ache, low back ache etc.

***Karppa vayu* & Pcos**

"பொருமிரத்தந்தனை மறித்துப் போதமிகவும் வலியுனண்டாங்க
குருதிசேரா வயிறுவலிபோங் கொள்ளுங் கர்ப்பந்தனை யழிக்கும்
வருடி யிடுப்புக் குடைந்துளைக்கும் மலத்தைமிகவும் மிறுக்கிக்கி
பெருகப் பனைக்கும் எனப் பெரியோர் பேசுங்கர்ப்பவாயுவிதே"

- அகத்தியர் ஆயுள்வேதம் 1200[35]

According to Siddha principles, the PCOS is a kind of *vayu* disorder called *karppa vayu* which is characterized by frequent miscarriages, abdominal pain, low back ache and severe constipation.

***Nirandhara maladu* & PCOS**

"நவின்றடவே யிடுப்புவயிற் பெருத்துக் காணும்
நலமான மேனியது வூதிக் காணும்
குவின்றிடவே மும்மடிப்பு வயிற்றில் தோன்றும்
குணவதியாந் தேவதா பெண்ணா னாலும்
நவின்றிடவே சன்மத்தின் மலடே யாகும்
சதாகாலங் கருப்பமது தரியா தென்று
புவின்றிடவே யூகிமுனி சிகிச்சா சாரம்
புகன்றிட்டார் லோகத்து மாந்தற் காமே"

- யூகி வைத்திய சிந்தாமணி[36].

Siddha Yugi muni in the above lines says that the symptoms of *Nirandhara maladu* are obesity, increased waist hip ratio, flabby abdomen with three folds of skin. He further says that females with these features may suffer from infertility. These symptoms can be correlated to PCOS in modern terms.

சூதக நோய் வரும்வழி:

"தரணியில் பெண்களுக்கு கெற்பநோய்கள்
நயக்கவே வந்து தென்னவென்றால் மைந்தா
நன்மையுடன் ருதுவாகும் நளிற்றானே
மயக்கவே மாப் பாண்டம் பால் பழத்தினாலே
வந்துதடா சூதகத்தின் வாயு தானே
தானென்ற கருக்குழியில் வாய்வு தங்கி
தளர்ந்த தொரு சோரையினால் தசைதான் மூடி
ஊனன்ற தேகமெல்லாம் மதர்த்து நல்ல
உண்மையுள்ள அடிவயிற்றில் வலியுண்டாச்சு
பானென்ற கருக்கழிதான் விளக்க மன்றி
பரமான விந்துவங்கே அணுகாதையா
ஏனென்றால் ஆதியிலே வாய்வு கொண்டு
இருந்ததினால் கெற்பமது இல்லை தானே"

- அகத்தியர் அமுத கலை ஞானம்(37)

The above lines explain the etiology of reproductive diseases according to Siddha science. It says consumption of high calorie diet like starchy foods, milk, fruits during menstruation results in accumulation of *Vayu* in the uterine cavity. This leads to reduced blood flow to the organ resulting obesity, abdominal pain and finally failure of conception.

"இசைந்ததொரு பெண்மலடு எங்குமில்லை
.....னாலே மலடான சேதிகேளு
அசைந்திருக்கும் பேயாலும் பித்தத்தாலும்
அடிவயிறு நொந்துவரும் வாயுவாலும்
பிசைந்தகர்ப்பப் புழுவாலும் கிரகத்தாலும்
பிணியாலும் மேகவை சூரியாலும்
துசங்கெட்டக் கலவியினால் பூவொதுங்கித்
துலங்காமற் பிள்ளையில்லை சொல்லக்கேளே"

- பதினெண் சித்தர்கள் பாடிய வைத்திய சில்லறைக் கோவை(38).

Naadi nadai:

"மாதர் கை மிடித்தபோது வந்திடும் நாடி மூன்றும்
சேதமாயிற்று நின்று சேரவே பதித்து நிற்கில்
ஓதுமே சூதகத்தில் ஓங்கிய வாய்வு நின்று
பேதமாய் வாதை பண்ணி பிணியினை விளைக்குந்த் தானே"

- அகத்தியர் அமுத கலை ஞானம்[39].

When the *naadi* is felt in women if all the 3 *naadi's* are found diffused and then felt together then those women are subjected to menstrual troubles and those women has *Soodhaga vaayu* and has different types of physical, characteristic and mental changes leads to problems.

3.2.2 MODERN ASPECT OF THE DISEASE

History:

Stein and Leventhal are regarded as the first investigators of polycystic ovary syndrome (PCOS), it was Vallisneri, an Italian medical scientist, physician and naturalist, who in 1721 described a married, infertile woman with shiny ovaries with a white surface and the size of ovaries as pigeon eggs [40]. Many scientists tried to explain the pathophysiology of PCOS and many studies were made. It is now accepted that it is multifactorial, partly genetic.

Other Names:

- Polycystic Ovarian Disease
- Functional Ovarian Hyperandrogenism
- Ovarian Hyperthecosis
- Sclerocystic Ovary Syndrome
- Stein-Leventhal Syndrome [41]

Definition:

Polycystic ovary syndrome (PCOS) is one of the most common heterogenous endocrinological and metabolic disorders in premenopausal women. Heterogeneous by nature, PCOS is defined by a combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses [42].

PCOS includes chronic non-ovulation and hyperandrogenaemia associated with normal or raised oestrogen (E_2), raised LH and low FSH/LH ratio.

PCOS is a problem with hormones that affects women during their childbearing years (age 15 to 55) age group have PCOS [43].

PCOS is a "syndrome", or group of symptoms that affects the ovaries and uterus. Its three main features are:

- Cysts in the ovaries
- High levels of male hormones
- Irregular or skipped periods.

The diagnosis and treatment of PCOS are not complicated, requiring only the judicious application of a few well-standardized diagnostic methods and appropriate therapeutic approaches addressing hyperandrogenism, the consequences of ovarian dysfunction and the associated metabolic disorders.

There are three recognized definition diagnose to PCOS, among The Rotterdam Criteria consider at least two of three criteria between clinical or biochemical hyperandrogenism, menstrual irregularity and polycystic ovaries characterized by ultrasound detection of 12 or more follicles [44].

In 1990 the National Institutes of Health (NIH) proposed that PCOS might be diagnosed with hyperandrogenism and irregular menstrual cycles without knowledge of ovarian ultrasound pattern [45].

Causes:

The exact cause of PCOS is unknown. This heterogenous is characterized by excessive androgen production by the ovaries mainly. PCOS is a multifactorial and polygenic condition. However, several studies have suggested that insulin resistance plays an important role in the pathogenesis of the syndrome. As a consequence of insulin-resistance, women affected by PCOS often present abnormalities of glucose metabolism and lipid profile and have an increased risk of type 2 diabetes and cardiovascular disease over-time [46].

Risk factors:

- Abdominal Adiposity
- Insulin Resistance
- Obesity
- Metabolic Disorders
- Cardiovascular disease

Pathogenesis:

Polycystic ovaries develop when the ovaries are stimulated to produce excessive amounts of male hormones (androgens), particularly testosterone, by either the release of excessive luteinizing hormone by the anterior pituitary gland, high levels of insulin in the blood (hyperinsulinemia) in women whose ovaries are sensitive to this stimulus or reduced levels of sex-hormone binding globulin (SHBG) resulting in increased free androgens.

The syndrome acquired its name due to the common sign on ultrasound examination of multiple ovarian cysts which represent immature follicles. The follicles have developed from primordial follicles but the development has stopped at an early antral stage due to the disturbed ovarian function. The follicles may be oriented along the ovarian periphery appearing as a 'string of pearls' on ultrasound examination. Patients with PCOS have higher Gonadotrophin releasing hormone (GnRH), which in turn results in an increase in LH/FSH ratio in females with PCOS. The majority of patients with PCOS have insulin resistance and/or obesity.

Their elevated insulin levels contribute to or cause the abnormalities seen in the hypothalamic-pituitary-ovarian axis that lead to PCOS. Hyperinsulinemia increases GnRH pulse frequency, LH over FSH dominance, increased ovarian androgen production, decreased follicular maturation and decreased SHBG binding. All these factors contribute to the development of PCOS.

PCOS is characterized by a complex positive feedback of insulin resistance and hyperandrogenism. In most cases, it cannot be determined which of those two should be regarded to be the causative agent. Experimental treatment with either anti-androgens or insulin sensitizing agents improves both hyper-androgenism and insulin resistance. Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol.

The excess of adipose tissue in obese patients causes them to have both excess androgens (which are responsible for hirsutism and virilization) and estrogens (which inhibit FSH via negative feedback). PCOS may be associated with chronic inflammation of the ovary which may induce conformational, endocrinal and metabolic changes which may predispose to PCOS. Several studies correlate the inflammatory mediators and oxidative stress with anovulation and other PCOS symptoms.

It was previously suggested that the excessive androgen production in PCOS could be caused by a decreased serum level of insulin-like growth factor binding protein-1 (IGFBP-1), in turn increasing the level of free IGF-1 which stimulates ovarian androgen production, but recent data concludes this mechanism to be unlikely. PCOS has also been associated with a specific fragile X mental retardation 1 (FMR1) sub-genotype. Many studies suggested that women who have heterozygous-normal/low FMR1 have polycystic-like symptoms of excessive follicle-activity and hyperactive ovarian function [(47)].

Relationship between PCOS and Insulin resistance:

- Insulin-resistance and hyperinsulinemia are tightly related to pathogenesis of PCOS which may be exacerbated by coexistence of obesity. Nevertheless, many studies showed that insulin resistance affected also normal weight women with PCOS. Besides, in lean patients with PCOS the visceral adiposity.
- PCOS also had impaired β cell function and the grade of dysfunction seems to be related to family history of type II diabetes. Furthermore, hyper insulinemic insulin resistance stimulates the ovarian androgen production and increases the likelihood to develop type 2diabetes, metabolic syndrome, and cardiovascular disease.

Relationship between PCOS and obesity

Obesity and insulin-resistance were significant risk factors to develop, the prevalence of type 2 diabetes. Dyslipidaemia is one of most frequent features in patients with PCOS. Hyperinsulinemia and insulin-resistance are related to abnormality of lipid profile, particularly to decreased levels of high-density lipoprotein cholesterol (HDL-C) levels and increased levels of triglycerides and of small dense low-density lipoprotein cholesterol (LDL-C).

Insulin-resistance causes a rise in free fatty acid (FFA) plasma levels due to increased synthesis from liver and increased mobilization from adipose tissue. The excess of FFA leads to insulin-resistance by inactivation of key enzymes such as pyruvate dehydrogenase (PDH) or by decreasing glucose transport activity, which may be a consequence of altered insulin signalling through decreased insulin receptor substrate-1 (IRS-1) associated PI3 kinase activity.

Clinical Manifestations:

Dermatological clinical manifestations of hyperandrogenism include:

Hirsutism:

Hirsutism defined as an excessive growth of terminal hair in androgen dependent areas of women. (Is one of the most widely used clinical criteria for the diagnosis of androgen excess and is observed in 50% - 80% of patients with hyperandrogenism)

Acne:

Acne is a disorder of the pilo sebaceous unit, with lesions on the face, neck, back and chest area. The importance of androgens in the acne pathogenesis is well-known and authenticated.

Androgenetic Alopecia:

The Androgenetic alopecia in women is characterized by hair loss in the central region of the scalp, with important psychosocial repercussions. Most patients with Androgenetic alopecia have the normal endocrine function.

Acanthosis nigricans:

Acanthosis nigricans characterized by the presence of a brown and velvety plate with accentuation in the furrows of skin. The dermatopathology is most commonly observed in the neck and intertriginous areas such as armpits, groin and infra mammary region and it is reported in 5% of patients PCOS seborrhoea, in severe cases, signs of virilization will present [(48)].

The symptoms of PCOS include:

- Irregular periods or absence of menstruation
- An increase in facial or body hair (hirsutism)
- Loss of hair on head
- Being overweight, experiencing a rapid increase in weight or having difficulty losing weight
- Oily skin, acne
- Difficulty becoming pregnant (reduced fertility).

Depression and psychological problems can also result from PCOS. The symptoms vary from woman to woman. Some women have very few mild symptoms, while others are affected more severely by a wider range of symptoms [(49)].

Diagnostic Criteria for PCOS:

NIH criteria:

In 1990, a workshop sponsored by the NIH suggested that a patient has PCOS if she has oligoovulation, signs of androgen excess (clinical or biochemical) and other entities are excluded that would cause polycystic ovaries.

Rotterdam criteria:

In 2003, a consensus workshop held in Rotterdam indicated PCOS to be present if any 2 out of 3 criteria including oligo ovulation and/or anovulation, excess androgen activity and polycystic ovaries (By gynaecologic ultrasound). The Rotterdam definition is wider, including many more patients, most notably patients without androgen excess. Critics say that findings obtained from the study of patients with androgen excess cannot necessarily be extrapolated to patients without androgen excess.

Table.No.1. Diagnostic Criteria for PCOS

S.no	Symptoms	NIH (1990) necessary criteria.	Rotterdam criteria (2003) necessary at least 2 criteria	Androgen Excess PCOS Society (2009) necessary at least criteria
1	Clinical hyperandrogenism or biochemical hyperandrogenism	Obligatory presence	Possible presence	Obligatory presence
2	Oligo / an ovulation may manifest with frequent or infrequent bleeding.	Obligatory presence	Possible presence	Possible presence
3	Ultrasound Polycystic Ovarian features presence of 12 or more follicles 2-9 mm in diameter.	-	Possible presence	Possible presence

Androgen excess PCOS Society criteria:

In 2006, the Androgen Excess PCOS Society suggested a tightening of the diagnostic criteria to all of the following including excess androgen activity, oligoovulation/ anovulation, polycystic ovaries and other entities are excluded that would cause excess androgen activity [(50)].

Differential Diagnosis:

- Hypothyroidism
- Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency)
- Cushing's Syndrome
- Hyper-Prolactinemia
- Androgen Secreting Neoplasms
- Pituitary or Adrenal Disorders [(51)]

Diagnosis:

- CT, USG

Laboratory Tests:

- Serum levels of androgens, including androstenedione and testosterone may be elevated. Dehydroepiandrosterone sulfate (DHEA-S) levels above 700-800 mcg/dl are highly suggestive of adrenal dysfunction. The free testosterone level is thought to be the best measure, with about 60% of PCOS patients demonstrating high levels.
- The ratio of LH (Luteinizing hormone) to FSH (Follicle stimulating hormone) is greater than 1:1 (sometimes more than 3:1), as tested on day 3 of the menstrual cycle.
- Fasting biochemical screening and lipid profile.
- Two hours oral glucose tolerance test (GTT) in patients with risk factors (Obesity, family history, history of gestational diabetes) may indicate impaired glucose tolerance (insulin resistance) in patients with PCOS].
- Fasting insulin level or GTT with insulin levels (also called IGTT): Elevated insulin levels have been helpful to predict response to medication and may indicate patients who will need higher doses of metformin or the use of a second medication to lower insulin levels. A hypoglycemic response in which the two-hour insulin level is higher and the blood sugar lower than fasting is consistent with insulin resistance.

May also include,

- Serum prolactin
- Serum androstenedione
- Pelvic ultrasound
- Basal body temperature monitoring
- Luteal phase progesterone measurement [52]

Complications:

Short term complications

- Menstrual irregularities
- Hyperandrogenism
- Infertility
- Obesity
- Pregnancy complication
- Obstructive sleep apnea
- Insulin resistance and hyperinsulinemia

Long term complications:

- Endometrial carcinoma
- Metabolic syndrome
- Cardio vascular disease
- T2DM / impaired glucose tolerance
- Nonalcoholic steatohepatitis
- Psychological disorders [53]

Treatment:

Classification of ovulation inducing drugs:

- Synthetic GnRH
- Human menopausal and chronic gonadotropins
- Bromocriptine
- Antiestrogenic compound

Other classification based on treatment:

- First-line treatment - Clomiphene citrate.
- Second-line treatments include - Gonadotropins.
  Laparoscopic ovarian surgery.
- Third-line treatment - In Vitro fertilization.

1. GnRH

Effects:

- Assessment of the function of the pituitary gonadotropins.
- Induction of ovulation.
- Treatment of males and females with idiopathic, hypothalamic, hypogonadotropic hypogonadism (Kallmann syndrome).

Adverse reactions:

Hot flushes, wearing, vaginal dryness, Headache, occasionally diminished libido and depression, prolonged treatment causes osteoporosis.

2. Human menopausal urinary Gonadotropin (hMG, Menotropins, Pregonal):

Effects:

Induce ovulation in women with anovulation due to pituitary-hypothalamic disorders but with normal ovaries and poly cystic ovaries.

Adverse reactions:

Hyperstimulation syndrome due to this the ovaries become very large and friable, abdominal pain, nausea, ascites and Shock.

3. Bromocriptine (Bromocriptine, Parlodel, Proctinal)

Effects:

It inhibits hyper prolactinemia and stops galactorrhoea. It lowers the serum prolactin levels and restores potency in males.

4. Antiestrogenic compound:

- **Clomiphene citrate**

Effects - Infertility treatment (ovulation induction)

Contraindications:

Allergy to Clomiphene – Pregnancy, hepatic disease, primary hypopituitarism, disturbed thyroid or adrenal function, uterine bleeding of unknown aetiology, hormone-dependent tumours.

Adverse effect:

Headache, vertigo, tiredness, disturbed vision, nausea, vomiting, vasomotor symptoms, facial flush, mastalgia, abdominal pain, paramania.

- **Metformin**

Effects - Restores regular bleeding and ovulation, reduces insulin resistance, improves arterial tension values, improves lipid profile, increases sex hormone binding globulin (SHBG) level may help reduce body weight.

Contraindications:

Hypersensitivity, renal insufficiency, acute or chronic diseases that may cause tissue hypoxia such as cardiac or respiratory insufficiency, lactation and hepatic damage.

Adverse effect:

Gastrointestinal disorders, lactic acidosis, dyspepsia, diarrhoea, nausea, flatulence.

- **Eflornithine**

Effects - Controls facial hirsutism

Contraindication:

Hypersensitivity to eflornithine or any adjuvant

Adverse effect:

Acne, chronic folliculitis barbae, alopecia, skin burning sensation, xeroderma, itching, erythema and skin formication

- **Spironolactone**

Effects: Inhibits androgens

Contraindications:

Hyperkalaemia, touch-sensitive nipples, mastalgia, menstrual disorders, hirsutism, agranulocytosis, headaches, sleepiness and ataxia.

Adverse effect:

Hypersensitivity, hyponatremia, hyperkalaemia, primary adrenal insufficiency, severe renal and hepatic failure and acute renal failure [54].

Laparoscopic surgery:

In women for whom CC or gonadotropin treatment is unsuccessful, another commonly used approach is ovarian surgery. Although there are several different techniques, they all involve acute ovarian tissue damage. Various types of ovarian surgery have been employed (wedge resection, electro cautery, laser vaporization, multiple ovarian biopsies and others). All of these procedures result in a positively altered endocrine profile after surgery.

Advantages of Surgery:

Direct visualization: The surgeon can directly see the structures on which she is operating.

***Observe nearby areas*:** In addition to treating the ovaries, the physician can visualize nearby structures and assess their health and condition, noting any other areas that may be of concern.

Disadvantages: Surgical Risks and Challenges:

***Anesthesia complications*:** Recent studies note concerns about neurotoxic effects on the brain and other areas of the body for patients who undergo one or more sessions of general anesthesia.

***Inadvertent enterotomy*:** When a patient has significant adhesions, it can be difficult for the doctor to see the structures beneath them. Thus, a surgeon can unintentionally cut into a nearby healthy organ or other structure – called an inadvertent enterotomy (IE). An IE can cause serious problems or death [55].

IVF (In Vitro Fertilization) [56]

The first 'IVF baby', Louise Brown, was born using natural IVF in 1978. Since then, the technology has advanced, and techniques refined in order to create safer and successful treatment.

Advantages of IVF:

IVF helps many patients who would be otherwise unable to conceive: The ultimate advantage of IVF is achieving a successful pregnancy and a healthy baby.

PCOS:

Polycystic ovary syndrome is common condition in which there is a hormone imbalance leading to irregular menstrual cycles. IVF has proved very successful in patients with PCOS, who will not conceive with ovulation induction.

There was a disadvantage are present in the IVF.

Diet Advice:

PCOS too needs a relatively strict lifestyle approach with regular exercise, movement and a high protein diet to help manage insulin levels and support weight loss.

Food to add:

- High-fiber vegetables, cruciferous vegetables, such as broccoli, cauliflower and Brussels sprouts
- Lean protein, such as fish
- Anti-inflammatory foods and spices, such as turmeric and tomatoes
- Greens, including red leaf lettuce and arugula and red peppers
- Beans and lentils
- Almonds
- Berries
- Sweet potatoes
- Winter squash

Food to avoid:

- Foods high in refined carbohydrates, such as rice, white bread and muffins
- Sugary snacks and drinks
- Inflammatory foods, such as processed a red meat
- Breakfast pastries
- Sugary desserts
- White potatoes
- Anything made with white flour [57]

***Siddha* drugs for PCOS:**

Chooranam:

- *Thratchathy chooranam* -1 to 2 gm twice a day with honey (5 ml)
- *Inji chooranam* - 1 to 2gm twice a day with water (50 ml)
- *Seenthil chooranam* -1 to 2 gm twice a day with hot water (5 ml)
- *Karisalai chooranam*-1 to 2 gm thrice a day with honey (5 ml)
- *Asoka pattai chooranam* -1 to 2 gm thrice a day with hot water (50 ml)
- *Attathi chooranam* - 1 to 2 gm twice a day with hot water (50 ml)
- *Thirikadugu chooranam* -1 to 2 gm twice a day with honey (50ml)
- *Meni Lavana Chooranam* - 1to 2 gm twice a day with honey(50ml)

Nei:

- *Bhrami nei*-10 to 15 ml at early morning
- *Senkottai nei*-15 ml twice a day
- *Venpoosani nei*-15 ml twice a day
- *Thanneervittan nei*- 10 ml twice a day

Manapagu:

- *Thurunchi manapagu*-10 to 20 ml twice a day with Luke warm water(50ml)
- *Madhulai manapagu*-10 to 15 ml twice a day with hot water

Ilagam:

- *Vilvathy ilagam*-5 to 10gm twice a day
- *Nellikai ilagam*-5 to 10gm twice a day
- *Karisalai ilagam*-5 to 10gm twice a day
- *Kumarai ilagam*-5 to 10gm twice a day
- *Thaneervittan ilagam*-5 to 10gm twice a day

Mezhugu:

- *Gunma kudori mezhugu*-500 to 1000 mg twice a day
- *Rasagandhi mezhugu*-300 mg twice a day with 5gm palm jaggery
- *Vaan mezhugu*-50 to 100 mg twice a day with 5gm palm jaggery
- *Rasa mezhugu*- 100 to 200 mg twice a day with 5gm palm jaggery
- *Nandhi mezhugu*- 100 to 200 mg twice a day with 5gm palm jaggery

Rasayanam:

- *Gandhaga rasayanam*-1.3 to 2gm twice a day

Maathirai:

- *Maha vasantha kusumaahara maathirai* (100 mg) - 1 to 2 mg twice a day with 10 ml leaf juice of karisalai
- *Sambirani poo guligai* (130 mg) - 1tab with sugar

Theeneer:

- *Lavana thiravagam* - 1 to 2 drops twice a day with water
- *Sanga thiravagam* - 1 to 2 drops twice a day with water

Parpam:

- *Kungiliya parpam*-100 to 300 mg twice a day with ghee (5 ml)
- *Muthu parpam*-30 to 60 mg twice a day with ghee (5 ml)
- *Muthuchippi parpam*-200 to 400 mg twice a day with ghee (5 ml)
- *Palagarai parpam*-65-130 mg twice a day with milk (50 ml)
- *Sangu parpam*-30 to 60 mg twice a day with ghee (5 ml)
- *Panja lavana parpam* - 30 to 60 mg twice a day with ghee (5 ml)

Chendooram:

- *Vediannabedi chendooram*-100 mg twice a day with hot water (50 ml)
- *Kalluppu chendooram*-488 mg twice a day with honey (5 ml)
- *Pattu karuppu*- 65 to 130 mg twice a day with honey (5 ml)
- *Kariyuppu chendooram*- 65 to 130 mg twice a day with honey (5 ml)
- *Aarumuga chendooram*-65 to 130 mg twice a day with honey (5 ml)
- *Ayakandha chendooram*-65 to 130 mg twice a day with honey (5 ml)
- *Aya chendooram* -65 to 130 mg twice a day with honey (5 ml)
- *Thalaga chendooram*-30 to 65 mg twice a day with 50 ml milk

Thylam:

- *Kalingathy thylam*-280 gm with 50 ml cold rice water from the first day of mensturation

Kuzhambu:

- *Kumatty kuzhambu*-130 mg with 5gm palm jaggery for 3 days from the first day of menstruation [58].

Yoga for PCOS:

- Pranayamam
- *Surya* Namaskaram (Sun Salutation), Bhujangasanam (Cobra Pose), Salabhasanam (Locust Pose), Dhanurasanam (Bow Pose), Trikonasana (Triangle Pose), Parsva -konasanam (Twisted Angle Pose), Sarvangasanam (Shoulder Stand), (Paschimottanasanam), fixed angle Pose, Savasanam[59].

3.3. PHARMACOLOGICAL REVIEW [60]

Animal models for the study of PCOS:

To identify the complex nature of this disease, suitable animal models are needed. Researchers during the past three decades have identified different animal models that mimic many of the features of PCOS in women. These models have afforded valuable information into complex nature of PCOS. Currently, although a genetic component to PCOS has been identified, a specific "PCOS gene" has not, and therefore a specifically targeted gene deletion for PCOS as an animal model is not available. Therefore, the majority of PCOS models that are available to date rely upon external chemical treatments with steroids, steroid precursors or steroid receptor antagonist to achieve the pathology. Many of these models do not produce consistent results or cease to produce PCOS like symptoms once the treatment stops. Recently, Hill et al. noted that their model for cardiovascular disease, the hypothalamic pro-opiomelanocortin (POMC) specific leptin-insulin double receptor knockout mouse exhibited some similar features to PCOS. Recently developed theca specific Esr1 specific knockout mouse model reproduces the clinical pathologic features of PCOS in 100% of animals.

Animal preparation:

Virgin, cyclic, adult female Wistar Albino rats (160–200 g) were employed for the study. Animals were acquired from Animal house. During the study all animals were caged in standard polypropylene cages and maintained in controlled environment

of (22 ± 3) C temperature, (55 ± 5) % humidity and a 12 h light/ dark cycle. They were fed with standard diet and water provided ad libitum. The study was duly approved by Institution Animal Ethics Committee (IAEC) for the use of animals and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Letrozole induced

Letrozole is an oral non-steroidal aromatase inhibitor. Inhibition of aromatase prevents the conversion of androgens to estrogens and therefore this model has similar features to the prepubertal androgen treatment. Immature rats (approximately 21 days old) are treated for 7-35 days with 400 μg/day letrozole. Similarly, to pre-pubertal androgen, this model is also reliant on artificial hyperandrogenaemia and does not help identify abnormalities upstream of hyperandrogenaemia.

Prenatal androgen

Prenatal androgen (PNA) treatment in sheep and monkeys' results in multiple metabolic and reproductive abnormalities. In monkeys, daily subcutaneous injections of 15 mg of testosterone propionate for 40-80 days gestation are needed to induce the syndrome. In ewes, an injection of 100 mg of testosterone propionate twice a week for 60 days between days 30 and 90 of the 147-day pregnancy result in the ovarian abnormalities. In both models, the abnormalities mirror the symptoms found in women with PCOS. These models produce a long-lasting effect in the female offspring mimicking many similar features of PCOS in humans. However, ewes and monkeys incur a large financial commitment for a long gestational period. However, it is noted that although PA monkeys exhibit hyperandrogenaemia, the increases are not as extreme as in PCOS women 0.3-0.4 ng/mL (~50-100% elevation above normal) ; PCOS women, 0.5-0.7 ng/mL (~70-200% elevation above normal) and that although anovulation observed in PNA monkeys, its prevalence is also significantly less than that of PCOS women (PA monkeys: ~40%; PCOS women ~90%).

Pre-pubertal androgen

This model exploits the association of elevated androgen levels during puberty and PCOS. Immature rats (approximately 21 days old) are treated for 7-35 days with ~100 μg/day testosterone propionate or dihydrotestosterone. Similar to the PNA animal

models, prepubertal androgen (PPA) animal models of PCOS utilize a unique window where administration of exogenous androgens results in permanent damage to the ovarian tissue and recapitulated the hallmark symptoms of PCOS in an animal model. PPA model shows many similar features to PCOS in women with the exception of the hallmark increase in basal LH levels. This model is reliant on artificial hyperandrogenaemia and therefore does not help identify abnormalities upstream of hyperandrogenaemia.

Dehydroepiandrosterone (DHEA) induced

Immature rats or mice (approximately 21-22 days old) are treated with daily DHEA injections (rats; 6 mg/100 g body weight, mice 6 mg/kg body weight) for 15-20 days. This dose of DHEA is sufficient to induce a hyper androgenized state similar to that in PCOS women. This model is also reliant on artificial hyperandrogenaemia and does not help identify abnormalities upstream of hyperandrogenaemia.

RU486 induced

Mature cycling female rats are treated daily with RU486 (20 mg/ kg body weight) for more than 8 days starting on the day of estrus. These animals exhibit increased basal LH, Polycystic ovaries, ovulation blockade and metabolic defects. However, this model is reversible and symptoms decrease upon cessation of the antiprogestin treatment.

Estradiol induced

The immune system is now a well-recognized component of reproductive biology. Immune cells are involved in all aspects of normal reproductive function including ovulation, corpus luteal formation, uterine receptiveness and maintenance of pregnancy. The recently developed PCOS model developed by Chapman and colleagues now provides evidence that aberrant immune function may play a role in the pathogenesis of PCOS and that PCOS may result from as autoimmune disease. This model exploits a 2-3-day window of neonatal period (5-7 days of age) when estradiol administration (20 μg/day) disrupts thymic maturation. As a consequence of this, increased vascular permeability in the thymus allows autoreactive T-cells to escape into the circulation. The ultimate effect of these “escapees” is a damage to tissues throughout the body including the ovaries. This model offers an abrupt change in the

direction of PCOS research. The major deficiencies in this model are a lack of hyperandrogenaemia and the fact that the loss of regulatory T-cell function would not only impact the ovary, but multiple other tissues resulting in pathologies not associated with PCOS. It will be interesting to follow the progress of this novel concept of autoimmune responses resulting in PCOS in women and if, PCOS women have deficiencies in regulatory T-cells.

Hypothalamic pro-opiomelanocortin (POMC) neuron specific leptin and insulin receptor KO

Hill and her colleagues were interested in insulin resistance and the development of type II diabetes when they developed their Pomc-Cre, Leproflox/flox IR flox/flox mice, effectively removing both leptin and insulin receptors specifically from the POMC. However, together with the anticipated glucose intolerance and insulin deficiency these mice suffer from hyperandrogenaemia and polycystic ovaries. These two pathological conditions secure its eligibility as a model for the study of human PCOS.

3.4. PHARMACEUTICAL REVIEW

Purification processes in *Siddha* system (*suddhiseithal)*

The process of detoxification or purification of the drug is called '*suddhiseithal*' in *Siddha* medical terminology.

Nature has created innumerable plant, herbs, metals, poisonous substances, minerals, salts and other organic substances. The Siddha had selected such of those things which can render relief to innumerable ailments of mankind suffered. Any matter in nature has to be utilized for medicine purposes the properties which may cause bad effects should be neutralized or eliminated. That's why every raw drug used in Siddha medicine is purified before preparing it as a medicine.

Purification

The exact part of the herb which has been prescribed should alone be taken for medicine. There should not be other impurities like mud, sand or any such thing. If it is green leaf, dried or decomposed leaves or insects should be eliminated. Care should be taken in identifying the herbs properly.

As a general rule, when anything is subjected to be processed by using heat, soaking either alone or with some other substance, some chemical reaction may take place. In these process impurities, toxins would be eliminated and the substances become purified. Hence some of the poisonous herbs which are purified by using heat.

Importance of purification

The drugs when subjected to heat like roasting or soaked in liquids undergo some chemical reactions. Such as oxidation of toxic substances to non-toxic substances, elimination of certain poisonous chemicals to non- poisonous substances. In these ways not only the toxicity, impurities or removed but also enhance the potency of the drugs.

Mezhugu (Waxy in nature) [(61)]:

Definition:

The drug that has to be made, the ingredients of it must be purified, by grinding or fusing attain mezhugu consistency.

This is of two types:

1. Araippu Mezhugu
2. Surukku Mezhugu

1. Araippu Mezhugu (obtained by grinding):

Mercurial compounds are ground individually or with other raw drugs adding juices or honey into a semisolid form.

2. Surukku Mezhugu:

Obtained by heating the mercurial compounds or paadaanas by adding oily substances or some juices drop by drop. After it is melting and attains semisolid form it should be placed in a mortar and tribute and then stored.

Storage:

Store it in a pure dry glass container.

Termination period:

Its termination period is 5 years.

What is Mezhugu?

Mezhugu is one of the 32 types of internal medicines described in *Siddha* system with a waxy consistency. Mostly it is a herbo-mineral preparation, consists of Mercury and mercurial salts, *Paadanam* (Poisonous), *Kaarasaaram* (Salts) and *Ubarasam* (Animal products) and processed with herbal juices. Most of the drugs in this type are made by the grinding process only. So, *Siddhars* like alchemy scientists has chosen the ingredients very carefully in the basis of '*Sathru*' (Antagonist), '*Mithru*' (Agonist) to nullify the toxicity and to potentiate the active principles of the drug respectively. These preparations are not chewable and given with the vehicle 'Palm jaggery'. Which is a good anti-dote for mercurial poisoning. Also, the drug is given as embedded in the vehicle to reach the gastric area without making contact in buccal cavity. Because, the buccal mucosa is highly sensitive to the drug reactions.

In another school of thought, Mezhugu is a one of the by product in the process of alchemy to attain the Gold as a goal. Almost all the *Siddhar's* were explaining about this type, So, the importance of this kind of drug is clearly exposed.

Table No.2. Few Other *Mezhugu* Names:

S.No	Drug name	Book Name	Page No
1.	இடிவல்லாதி	புலிப்பாணி வைத்தியம் 500	130-131
2.	இரசகந்திமெழுகு	புலிப்பாணி வைத்தியம் 500	132-134
3.	கந்திமெழுகு	சித்த வைத்தியத் திரட்டு	134
4.	நந்திமெழுகு	சித்த வைத்தியத் திரட்டு	135-139
5.	குன்ம குடோரி	சித்த வைத்தியத் திரட்டு	134-135
6.	நவஉப்பு மெழுகு	சித்த வைத்தியத் திரட்டு	139-140
7.	பஞ்சசூத மெழுகு	யூகி கரிசல் – 151	141-142
8.	மகாவீர மெழுகு	சித்த வைத்தியத் திரட்டு	142-143
9.	மலக்குடார மெழுகு	சித்த வைத்தியத் திரட்டு	143
10.	இந்துவர்ண மெழுகு	யூகி கரிசல் – 151	143-144

Table No.3. Analytical Specification of Avaleha / Leham / Ilagam (Confections / Semi solid)

S.NO	TESTS
1.	Description
	Colour
	Odour
	Taste
2.	Loss on drying at 105^0C
3.	Total – ash
4.	Acid - insoluble ash
5.	Ph
6.	Total solid
7.	Fat content
8.	Reducing sugar
9.	Total sugar
10.	Identification, TLC/HPTLC
11.	Test for heavy metals Lead Cadmium Mercury Arsenic
12.	Microbial contamination Total bacterial count Total fungal count
13.	Test for specific pathogen *E. coli* *Salmonella spp.* *S. aureus* *Pseudomonas aeruginosa*

14.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
15.	Test for Aflatoxins (B_1, B_2, G_1, G_2)

3.5. LATERAL RESEARCH OF THE DRUG

1. Borax:

Antidote activity

Evaluation of processed borax as antidote for aconite poisoning

Results

Protection index indicates that treated borax gave protection to 50% rats exposed to the lethal dose of toxicant in acute protection Study. The ventricular premature beat and ventricular tachyarrhythmia caused by the toxicant were reversed by the test drug indicate reversal of toxicant induced cardio-toxicity. The acetylcholine induced contractions in leech muscle were inhibited by toxicant and it was reversed by test drug treatment.

Conclusion

The processed borax solution is found as an effective protective agent to acute and sub-acute aconite poisoning, and aconite induced cardiac and neuro-muscular toxicity. Processed borax at therapeutic dose (22.5 mg/kg) has shown better antidotal activity profile than five times more than therapeutic dose (112.5 mg/kg) [62].

2. *Commiphora myrrha:*

Antimicrobial activity of *Commiphora myrrha*

The activity of *commiphora myrrh* against microorganisms recovered from wounds

Extracted, purified, and characterized 8 sesquiterpene fractions from *C. molmol* were a mixture of furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, which showed antibacterial and antifungal activity against standard pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, with minimum inhibitory concentrations ranging from 0.18 to 2.8 mg/ml.

These compounds also had local anaesthetic activity, blocking the inward sodium current of excitable mammalian membranes. (Dolara *et al,* 2000).

The antifungal activity of Myrrha *(Commiphora molmol)* essential oil was tested against three s fungi, *Aspergillus flavus, Aspergillus Niger* and *Penicillium citrinum*, Results showed inhibitory effect of the oil against tested fungi with the increasing concentrations. Minimum inhibitory concentration (MIC) using agar dilution method was 4% (v/v) for *A. Niger* and *A. flavus* and above 4% (v/v) for *P. citrinum* (Batool, 2007).

Conclusions

Two hundred clinical isolates were obtained from wounds swab after bacteriological procedure, antimicrobial test of *Commiphora myrrh* were subjected by tube dilution method technique against Gram Negative and Gram-Positive organisms. This study concluded that:

A. The most common pathogen isolated positive cocci from wounds was S*taphylococcus aureus.*
B. *Commiphora myrrh* is suitable antimicrobial agent against *Staphylococcus aureus.*
C. Medicinal plants remain as a potential source of antimicrobial agents [(63)].

3. Crocus sativus:

Antidiabetic potential of saffron and its active constituents

The prevalence of diabetes mellitus is growing rapidly worldwide. This metabolic disorder affects many physiological pathways and is a key underlying cause of a multitude of debilitating complications. There is, therefore, a critical need for effective diabetes management. Although many synthetic therapeutic glucose-lowering agents have been developed to control glucose homeostasis, they may have unfavourable side effects or limited efficacy. Herbal-based hypoglycaemic agents present an adjunct treatment option to mitigate insulin resistance, improve glycaemic control and reduce the required dose of standard antidiabetic medications. Saffron (*Crocus sativus* L.), whilst widely used as a food additive, is a natural product with insulin-sensitizing and hypoglycaemic effects. Saffron contains several bioactive β carotenes, which exert their pharmacological effects in various tissues without any obvious side effects. In this study, we discuss how saffron and its major components exert their hypoglycaemic effects by induction of insulin sensitivity, improving insulin

signalling and preventing β-cell failure, all mechanisms combining to achieve better glycaemic control [64].

Evaluation of *Crocus sativus* L. Stigma Phenolic and Flavonoid Compounds and Its Antioxidant Activity

Antioxidant activity:

The antioxidant activity of saffron could be attributed to two bioactive compounds, crocin and safranal, and a DPPH radical scavenging test on crocin and safranal exhibited antioxidant activities of 65% and 34%, respectively, at 500 ppm. In this study, the antioxidant activity of the methanolic extract at 300 μg/mL was 68.23%.

Conclusion:

Saffron stigmas were found to possess antioxidant activity, hence, saffron is a promising natural product in this respect. Different solvents affected the total phenolic and flavonoid contents of the extracts and led to the observation of different antioxidative efficacy. The gallic acid and pyrogallol as bioactive compounds present in saffron stigma contributed in its antioxidant activity. It is suggested that saffron stigma besides being colorant could play a role as antioxidant source, which might enhance the quality of the products in functional foods, beverages, drinks, pharmaceutical and cosmeceutical industries [65].

Effects of saffron (*Crocus sativus* l. Iridaceae) on blood level of follicle-stimulating hormone, and number and dynamics of body weight of offspring in female rats.

This paper reviews the effects of saffron Crocus sativus on blood level of follicle-stimulating hormone (FSH), and number and dynamics of body weight of offspring in female rats. The findings indicated that the per os administration of alcoholic extract of saffron was able to decrease the FSH levels in blood of the 12-month-old rats as compared to that in the control group, involving the animals of the same age which have not received the saffron extract, and was close to the FSH levels reported for the 6-month rats. There was also an increase in number and body weight of pups from rats receiving the saffron extract prior to pairing with the intact males [66].

4. *Syzygium aromaticum:*

Anti -cancer activity:

Eugenol treated HL-60 cells displayed features of apoptosis including DNA fragmentation and formation of DNA ladders in agarose gel electrophoresis. Eugenol transduced the apoptotic signal via ROS generation, there by including MTP, reducing anti-apoptotic protein bcl-2 levels, inducing cytochromes c release to the cytosol, and subsequent apoptotic cell death [(67)].

Antimicrobial and antioxidant activity of unencapsulated clove (*Syzygium aromaticum*, L.) essential oil

Clove (*Syzygium aromaticum*, L.) essential oil is known for its antimicrobial activity against several pathogenic bacteria. Encapsulation of clove oil was proposed as a mean to disguise its strong odour that limits its uses in food industry. Thus, the aim of this study was extraction, encapsulation and assessment of the antimicrobial and antioxidant potential of clove essential oil. The essential oil showed high DPPH scavenging capacity and low hydroxyl radical inhibition. Clove essential oil showed *in vitro* inhibitory and bactericidal effect against *S. aureus, E. coli, L. monocytogenes* and *S. aureus* was superior to nitrite. The essential oil particles encapsulated with sodium alginate and emulsifiers, showed high encapsulation efficiency, low antioxidant activity and strong antimicrobial inhibition. Similar bacterial growth was observed in meat-like products after addition of either particles or nitrite [(68)].

Phenolic extracts of clove (*Syzygium aromaticum*) with novel antioxidant and antibacterial activities

Introduction

Clove (*Syzygium aromaticum*) is a rich source of bioactive compounds. The goal of this study was to test different extracts of clove in terms of their phenolic contents, their antioxidant potential and their antibacterial action against pathogenic bacteria.

Results

Antioxidant potential of clove extracts was estimated using DPPH$^{\bullet}$ (1,1-diphenyl-2 picrylhydrazyl), ABTS$^{\bullet+}$ 2, 2′azino bis-(3-ethylbenzthiazoline-6-sulfonic acid), β-carotene-linoleic bleaching assay and ferric reducing antioxidant power

(FRAP). Ethanol and water extracts showed comparable antioxidant activity to the synthetic antioxidant *tert*-butylhydroquinone (TBHQ). The DPPH$^{\bullet}$ radical quenching activity varied from 25.3 to 91.4%, while clove extracts showed ABTS$^{\bullet+}$scavenging activities from 49.4 to 99.4%. Clove extracts inhibited the bleaching of β-carotene wherein the order of decreasing activity was water > ethanol > ethyl acetate extracts as compared with TBHQ. Clove extracts exhibited antibacterial activities against the growth of *S. aureus* and *E.coli* in concentration range from 50 to 100 μg/mL[(69)].

4. MATERIALS AND METHODS

Selection of the Drug:

For this present study, herbo-mineral formulation of ***Soodhaga mezhugu*** was taken as a compound drug preparation for *Soodhaga vayu* has been taken from the *Siddha* classical literature **"*Kosayee Anuboga Vaithiya Biramma Ragasiyum*"** written by ***Munusamy mudaliyar.*** Page No-227[70].

Ingredients of the drug:

- *Vengarathool [Sodium biborate]*
- *Valendhrapolam [Commiphora myrrha]*
- *Kunguma poo [Crocus sativus]*
- *Kirambu thylum [Syzygium aromaticum]*

Collection of the raw materials:

The raw drugs are *Vengarathool, Valendhrapolam* and *kunguma poo* were bought from Ramasamy Chetty country shop at Parrys, Chennai.

Kirambu thylum was prepared from Government Siddha Medical College, Pharmacy (Department of Gunpadam), Arumbakkam, Chennai.

Identification and authentication:

All raw drugs were identified and authenticated by the experts of *Gunapadam* (Pharmacology) at Government Siddha Medical College, Arumbakkam, and Chennai.

The specimen samples of the identified raw drugs were preserved in the laboratory of P.G *Gunapadam* for future references.

Purification: [71]

Purification process was done as per classical Siddha literature **"*Gunapadam Thathu seeva vaguppu*".**

1.Purification of *Vengaram* (*Sodium biborate*):

Roasted in a pan till the moisture completely evaporates.

2. Purification of *Valendhrapolam (Commiphora myrrha)* [72]*:*

Valendhrapolam is boiled in vinegar steam.

3. Purification of *Kunguma poo* (*Crocus sativus):*

The unwanted materials are removed.

4. Purification of *Kirambu* (*Syzygium aromaticum):*

Removed all the impurities and dried it in the sunlight.

4.1. PREPARATION OF THE *SOODHAGA MEZHUGU*:

- *Vengarathool [Sodium biborate]* - 975 mg (7 ½ kundri)
- *Valendhrapolam [Commiphora myrrha]* - 780 mg (6 kundri)
- *Kunguma poo* [*Crocus sativus]* - 205 mg (1 ½ kundri)
- Kirambu thylum [*Syzygium aromaticum]* - 1 drop

Procedure:

- Purified *Vengaram* is placed in a stone mortar and ground. Then *Kunguma poo* is added with the above powder and grounded. Purified *Valendhrapolam* and few drops of *Kirambu thylum* is mixed with the above paste and triturated for about 4 hours until get the waxy consistency. The *Mezhugu* is obtained and stored in a closed air tight container.

Preservation:

The medicine was preserved in well stoppered air tight glass containers and labelled as ***SM* (*Soodhaga Mezhugu*).**

Drug profile:

Administration of the drug

Form of the medicine	- Wax
Route of administration	- Enteral (Oral)
Dose	- 0.202 gm (Thuvarai alavu)
Adjuvant	- Palm jaggery
Shelf life of the *Mezhugu*	- 5 years
Indication	- ***Soodhagha vayu*** **(PCOS).**

Fig.No.6. Ingredients of *Soodhaga Mezhugu*:

1.Vengarathool (Sodium biborate):

Unpurified

Purified

2. *Valendhrapolam (Commiphora myrrha):*

Unpurified

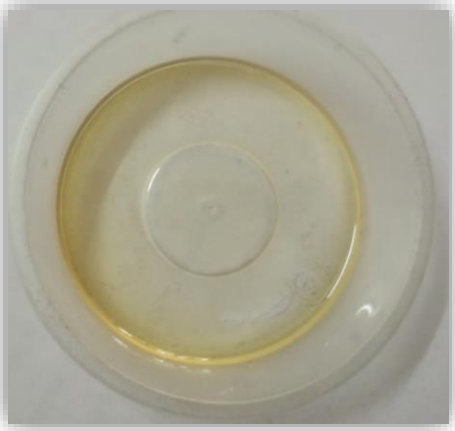
Purified

3. *Kunguma poo* (*Crocus sativus):*

Unpurified

Purified

4. Kirambu thylum (Syzygium aromaticum):



Clove oil

Fig.No.7. Preparation of *Soodhaga Mezhugu*:

Preparation of *Soodhaga Mezhugu*

Step 1

Step 2

Final product of *Soodhaga Mezhugu*

4.2. STANDARDIZATION OF THE *SOODHAGA MEZHUGU*:

Standardization of drug means confirmation of its identity, determination of its quality, purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations.

Method of standardization:

Techniques Involved in Standardization of Compound Drugs:

- Macroscopic Methods
- Microscopic Methods
- Physical Methods
- Chemical Methods
- Biological Methods

4.2.1. ORGANOLEPTIC CHARACTER OF THE *SOODHAGA MEZHUGU* [73]:

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its color, odour, taste, texture etc.

Colour:

A sample of *Mezhugu* was taken in watch glasses and placed against white back ground in white tube light. The *Mezhugu* was observed for its color by naked eye.

Odour:

Mezhugu were smelled, the time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste:

A sample of *Mezhugu* was tasted and the taste was reported.

Results were noted and tabulated in Table. No:7.

4.2.2. PHYSICO-CHEMICAL ANALYSIS [74]:

Physico-chemical studies like total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, Dr. MGR University, Chennai.

Solubility Test:

A few gram of sample *(SM)* was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

pH value:

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the ***Soodhaga mezhugu*** was written in results column.

Loss on Drying:

An accurately weighed 1gm of ***Soodhaga mezhugu*** formulation was taken in a tarred glass bottle. The crude drug was heated 105°c for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

Determination of total Ash:

Weighed accurately 2g of ***Soodhaga mezhugu*** formulation was added in crucible at a temperature 600°c in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air-dried drug.

Determination of acid insoluble ash:

Ash above obtained was boiled 5min with 25ml of 1M hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air-dried drug.

Determination of water-soluble ash:

Total Ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with water and ignited for 15 min at a temperature not exceeding 450°c in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

Determination of water-soluble extractive:

5gm of air-dried drug. Coarsely powered ***Soodhaga mezhugu*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 100°c and weighted. The percentage of water-soluble extractive was calculated with reference to the air-dried drugs.

Determination of alcohol soluble extractive:

1gm of air-dried drugs, coarsely powdered ***Soodhaga mezhugu*** was macerated with 20ml. Alcohol in closed flask for 24 hours. With frequent shaking.it was filtered rapidly talking precaution against loss of alcohol .10ml of filtrate was the evaporated in a tarred flat bottom shallow dish, dried at 100°c and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug. Results were noted and tabulated in Table. No:8.

4.2.3. PHYTO-CHEMICAL EVALUATION [(75)]:

Phytochemical are chemical compounds that are naturally present in plants. Phytochemical screening of the plant gives a vast idea about the chemical constituents present in the drug.

The ***SM*** was subjected to the following phytochemical screening.

Preparation of the extract

The preliminary phytochemical screening test was carried out for each extract of ***Soodhaga mezhugu*** as per the standard procedures.

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. Detection of Carbohydrates:

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch' Test: To 2ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

3. Detection of Glycosides:

Extracts were hydrolyzed with dil. HCL, and then subjected to test for glycosides.

a) Modified Borntrager's Test:

Extracts were treated with Ferric chloride solution was added and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was again separated and treated with ammonia solution. Formation of rose-pink color in the ammoniacal layer indicates the presence of anthranol glycosides.

4. Detection of Saponins:

a) Foam Test: 0.5 gm of extract was shaken with 2 ml of distilled water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of Phenols Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

6. Detection of Tannins Gelatin Test:

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

7. Detection of Flavonoids:

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

8. Detection of Proteins:

a) Xanthoprotein test: The extracts were treated with few drops of cone. Nitric acid. Formation of yellow color indicates the presence of proteins.

9. Detection of Amino acids:

a) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

10. Detection of Diterpenes Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

11. Gum and Mucilage:

To 1 ml of extract add 2.5 ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicates presence of gum and mucilage.

12. Test for Quinones:

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

13. Test for Fixed oils and Fats:

a) Spot Test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils. Results were noted and tabulated in Table. No:9.

4.2.4. BIOCHEMICAL ANALYSIS [76]:

The bio-chemical analysis was done to identify the acid and basic radicals present in the sample.

Preparation of extract

5g of *SM* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This method is used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

Preliminary Basic and Acidic radical studies

Preparation of extract

5gm of *SM* was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 20 minutes. Then it was cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water.

Table. No.4. Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
1.Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow color precipitate.	Presence of Potassium.
2.Test for Calcium: To 2 ml of the *SM* extract 2 ml of 4% ammonium oxide solution was added.	Formation of White precipitate.	Presence of Calcium.
3.Test for Magnesium: To 2ml of *SM* extract, drops of sodium hydroxide solution was added.	Formation of White precipitate	Presence of Magnesium.
4.Test for Ammonium: To 2ml of *SM* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown color.	Appearance of brown color.	Presence of Ammonium.

5. Test for Sodium: Hydrochloric acid was added with a pinch of the *SM* sample and a paste was made and introduced into the blue flame of Bunsen burner.	Appearance of intense yellow color	Presence of Sodium.
6. Test for Iron (Ferrous): The *SM* extract was treated with Conc. HNO3 and ammonium thiocyanate.	Appearance of blood red color.	Presence of Iron.
7. Test for Zinc: To 2 ml of the *SM* extract drops of sodium hydroxide solution was added.	Formation of White precipitate.	Presence of Zinc.
8.Test for Aluminium: To the 2m1 of the *SM* extract sodium hydroxide was added in drops and noted.	White precipitate obtained	Presence of Aluminium.
9. Test for Lead: To 2 ml of *SM* extract 2ml of potassium iodide solution was added and noted.	Formation of Yellow color precipitate.	Presence of Lead.
10. Test for Copper: a. A pinch of *SM* sample was made into a paste with concentrated. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted. b. To 2 ml of *SM* extract excess of ammonia solution was added.	a & b. Appearance of Blue color	Presence of Copper.

11. Test for Mercury: To 2m1 of the *SM* extract sodium hydroxide solution was added.	Formation of Yellow precipitate.	Presence of Mercury.
12. Test for Arsenic: To 2 ml of the *SM* extract 2ml of Sodium hydroxide solution was added.	Formation of brownish red precipitate	Presence of Arsenic.

Results were noted and tabulated in Table. No:10.

Table. No.5. Test for acid radicals

PROCEDURE	**OBSERVATION**	**INFERENCE**
1. Test for Sulphate: To 2 ml of the *SM* extract 5% of barium chloride solution was added.	Appearance of White precipitate.	Presence of Sulphate.
2. **Test for Chloride:** The *SM* extract was treated with silver nitrate solution and observed.	Appearance of White precipitate.	Presence of Chloride.
3.Test for Phosphate: The *SM* extract was treated with ammonium molybdate and concentrated HNO3 and observed.	Appearance of Yellow precipitate.	Presence of Phosphate.
4.**Test for Carbonate**: The *SM* extract was treated with concentrated Hcl and observed. for the appearance of effervescence.	Formation of effervescence.	Presence of Carbonate.

5.Test for Fluoride & Oxalate: To 2ml of extract 2ml of dil. acetic acid and 2ml calcium chloride solution were added and heated.	Formation of cloudy appearance.	Presence of Fluoride & Oxalate.
6. Test for Nitrate: To 1 gm of the *SM*, copper turnings were added and again concentrated H2SO4 was added, heated and the test tube was tilted vertically down.	Characteristic changes.	Presence of Nitrate.

Results were noted and tabulated in Table. No:11.

The bio-chemical analysis was done to identify the acid and basic radicals present in the *SM.*

4.2.5. TLC/HPTLC finger print studies [(77)]

HPTLC finger printing was carried out as per the reference.

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated Sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample Applicator : Camag Linomat - IV applicator with N_2 gas flow.

Photo documentation System : Digi store - 2 documentation system with Win Cat & Video scan software.

Scanner : Camag HPTLC scanner - 3 (030618), Win Cats - IV.

Development Chamber	: Camag HPTLC 10X10, 10 X 20 twin trough linear Development chamber.
Quantity applied	: 5, 10 µl for extracts and 5 µl for standards
Stationary phase	: Aluminum plate pre-coated with silica gel 60(E. Merck)
Plate thickness	: 0.2 mm.
Mobile Phase	: For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract - Toluene: Ethyl acetate (1:1).
Scanning wavelength	: 254 nm
Laboratory condition	: 26 ± 5^{o}C and 53 % relative humidity

The plate was developed up to a height of 8cm, air dried, spots were observed under the UV light at 254 and 366nm. Finally, the plates were derivatized using Vanillin-Sulphuric acid reagent heated at 105^{0} till color spots appeared. Results were noted and tabulated in Table. No:12, 13, Graphs. No:1, 2.

4.2.5. ANTIMICROBIAL ACTIVITY [(78)]:

AGAR- WELL DIFFUSION METHOD

The plate count technique is one of the most routinely used procedures because of the enumeration of viable cells by this method.

PRINCIPLE

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

1. *E. coli* (ATCC 25922)
2. *Staphylococcus aureus* (ATCC 25923)
3. *Pseudomonas aeruginosa* (ATCC 27853)
4. *Klebsiella pneumoniae* (ATCC 13883)

PROCEDURE

Petri plates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae a*nd *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control. Results were noted and tabulated in Table. No:14.1, 14.2, 14.3, 14.4.

ANTIFUNGAL ACTIVITY

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

MATERIALS REQUIRED

1. Potato Dextrose Agar Medium (1 L)

The medium was prepared by dissolving 39 g of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Clotrimazole (standard antifungal agent, concentration: 10mg / ml)

3. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

- *Aspergillus niger* (ATCC 16404*)*

PROCEDURE

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The zone of inhibition was measured after vernight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993). Results were noted and tabulated in Table. No:14.5.

4.2.7. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-red Spectroscopy [(79)]

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterize some inorganics. Examples include paints, adhesives, resins, polymers, coatings and drugs. FTIR is an effective analytical instrument for detecting functional groups.

Applications:

- Quantative scans
- Qualitative scan solids, liquids, gases
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation
- Pharmaceuticals

Fig.No.8. FTIR INSTRUMENT

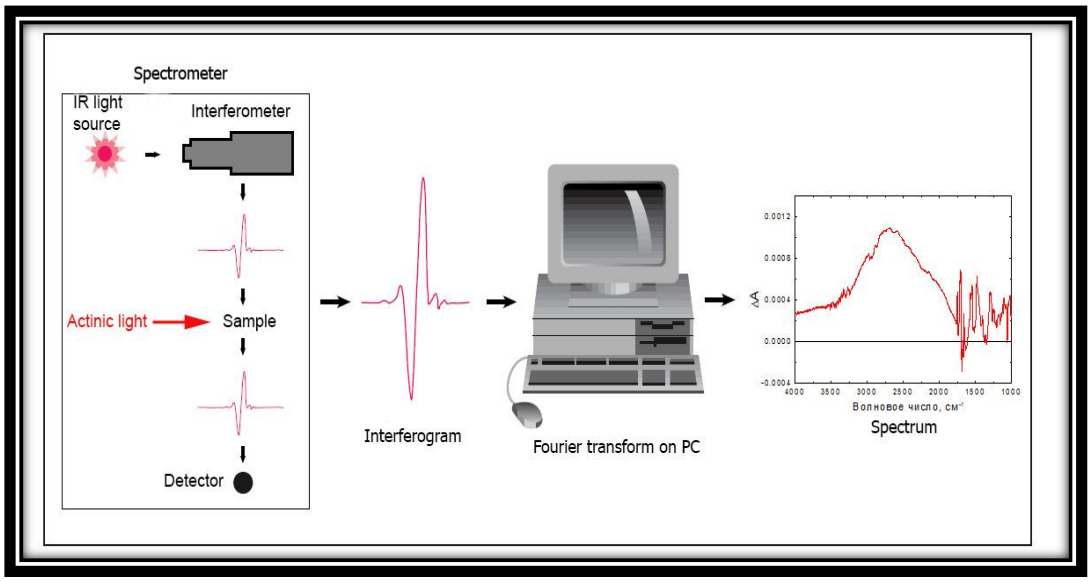


Fig.No.9. FT IR MECHANISM

Principle:

Spectrophotometric tests are commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures are applicable to substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›):

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable die.
- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10%

between them (For e.g. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

Results were noted and tabulated in Table. No:15, Graph. No:3.

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig.No.10. ICP-OES INSTRUMENT

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP).

Principle:

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000– 10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analyzed by ICP-OES. [80]

Application:

The analysis of major and minor elements in solution *SM*.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms

Mechanism:

In plasma emwassion spectroscopy (OES), a *SM* solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values.[81]

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

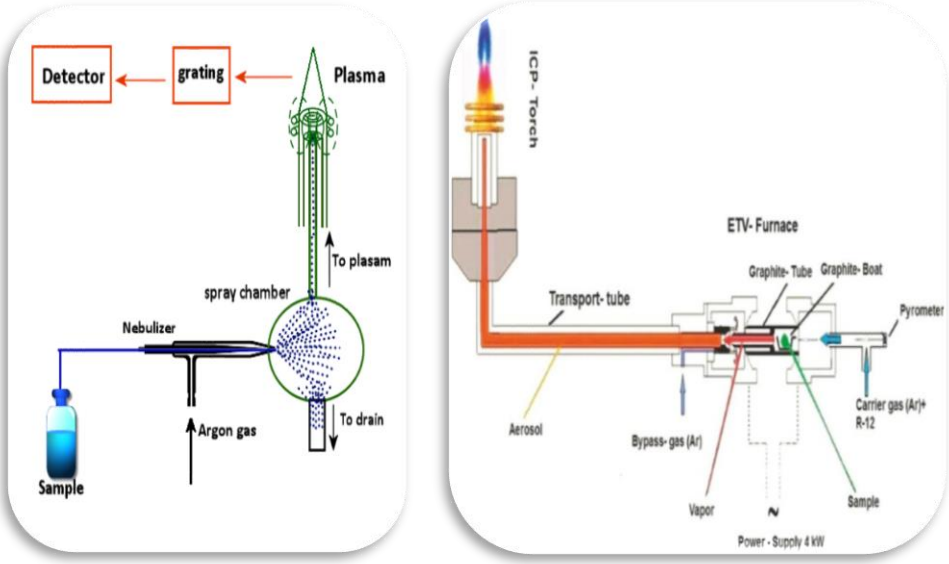


Fig No.11. ICP-OES MECHANISM

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby *SM* are introduced in liquid form for analysis.

100 mg *SM* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested *SM* solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106. Results were noted and tabulated in Table. No:16.

SEM - Scanning Electron Microscope [82]:

Definition:

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM analysis applications

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture)

- Chemical composition (when used with EDS) Orientation of materials making up the sample

The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal

SEM Analysis with EDS – qualitative and semi-quantitative results

Magnification – from 5x to 300,000x

Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height

Materials analyzed – solid inorganic materials including metals and minerals.

Fig.No.12.SEM INSTRUMENT

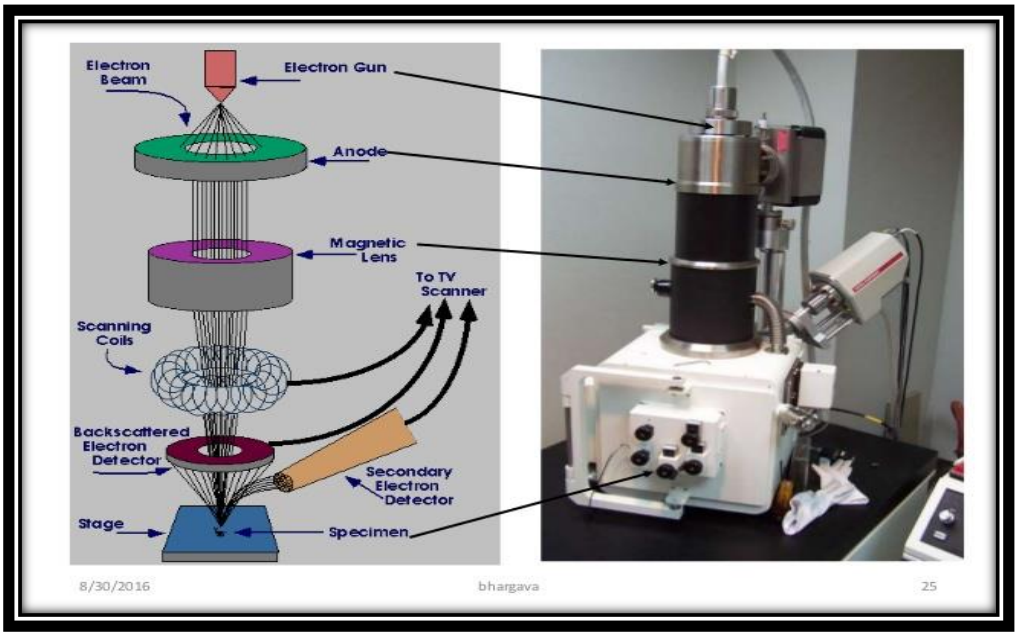


Fig.No13.SEM MECHANISM

The SEM analysis process

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyze the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam was focused through a probe towards PP. Variety of signals was produced on

interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include:

- Secondary electrons
- back scattered electrons
- characteristic x-rays light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample. Results were noted and in Figure.No:17, 18.

XRD - X-ray Powder Diffraction (XRD) [(83)]

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

Definition

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

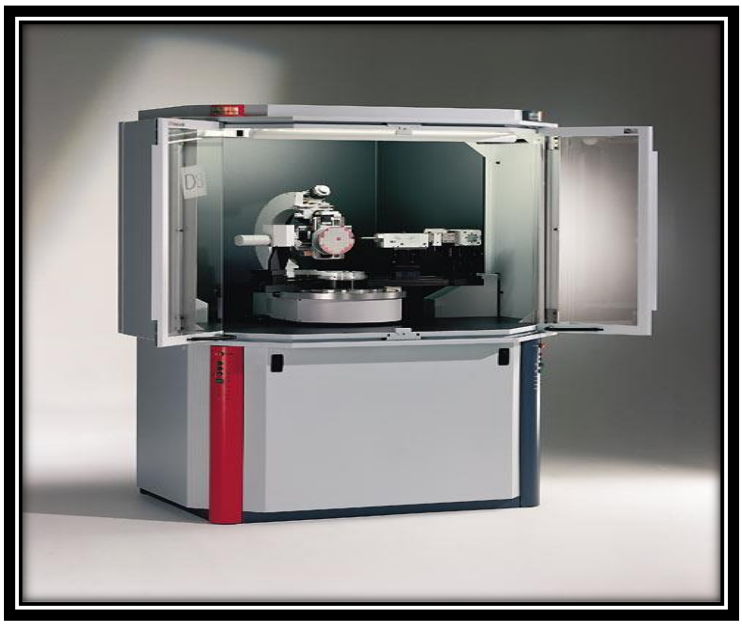


Fig.No.14. XRD - X-ray Powder Diffraction

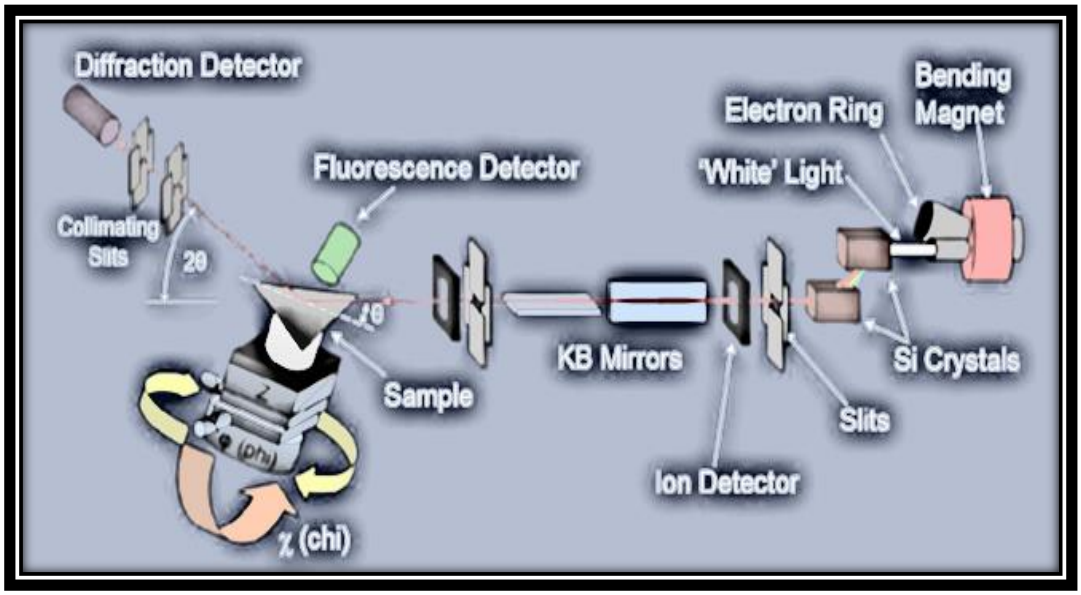


Fig.No.15. XRD Mechanism

Applications:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to:

- Determine crystal structures using Riveted refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
- determining lattice mismatch between film and substrate and to inferring stress and strain
- determining dislocation density and quality of the film by rocking curve measurements
- measuring super lattices in multilayered epitaxial structures
- determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations:

- Homogeneous and single-phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 μm (or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface.

Results were noted and in Graph. No:4.

4.3. TOXICOLOGICAL STUDY:

4.3.1. ACUTE ORAL TOXICITY – OECD GUIDELINES – 423[84]

- Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423)
- The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA **(IAEC approved Number: 01/321/PO/Re/S/01/CPCSEA dated 12/10/2018.)**
- These studies were conducted in C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai**.**

Introduction:

- The acute toxic class method was stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.

- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.

- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

PRINCIPLE

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of Rats per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental Rats at one of the defined doses. The substance is tested using a stepwise procedure, each step using three Rats of a single sex. Absence or presence of compound-related mortality of the Rats dosed at one step will determine the next step, i.e.; − no further testing is needed − dosing of three additional Rats with be the same dose − dosing of three additional Rats at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

METHODOLOGY

Selection of Rats

- The animal models used in this study were albino Rats.
- Healthy Female albino Rats weighing 150-250gm were obtained from the animal house of Kings Institute, Guindy, Chennai.
- Females should be nulliparous and non-pregnant.
- Each animal must be around 8 and 12 weeks old at the time of dosing.
- The studies were conducted in the animal house of C.L. Baid Metha college of Pharmacy, Chennai-97.

Housing and Feeding Conditions

- Rats were housed under standard laboratory conditions.
- They were maintained in a ventilated room. The temperature in the room should be 22° (±3°).
- The relative humidity should be at least 30% and not exceed 70% (50%-60%).
- Lighting should be artificial; it is maintained as 12h light/dark cycle.
- Rats were kept in a clean polypropylene cage.
- Rats were fed with standard pellet diet (Biogen Foods, Bangalore) and water *ad libitum*.

Preparation of Rats

All the Rats were randomly selected and marked on its fur for its individual identification. They were acclimatized to the laboratory conditions at least one week prior to the commencement of the study.

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the ***Soodhaga mezhugu.***

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design.

IAEC approved Number	**:**01/321/PO/Re/S/01/CPCSEA dated 12/10/2018.
Test Substance	: ***SOODHAGA MEZHUGU***
Animal Source	: The animal house of Kings Institute, Guindy, Chennai.
Animals	: Wistar Albino Rats (Female-3+3)
Age	**:** 8 to 12weeks
Body Weight on Day 0	**:** 150-250gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** Animals were kept in individual cage and numbered.

Number of animals	: 3 Female/group,
Route of administration	: Oral
Feed and Water	: Rodent pellet feed (Sai Meera) purified water libitum.
Housing & Environment	**:** Individually in polypropylene cages
Housing temperature	**:** Between 22ºC + 3ºC.
Relative humidity	**:** Between 30% and 70%,
Air changes	**:** 10 to 15 per hour and
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**:** 48 hours.
Evaluation	**: 14 days.**

EXPERIMENT PROCEDURE

Administration of doses

Soodhaga Mezhugu prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of albino Rats. It is given in a in a single oral dose by gavage using a feeding needle. Rats were fasted prior to dosing. Following the period of fasting, the Rats were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 h and these Animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of Animals and dose levels

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 Rats (3 Rats per step).

Duration of Study : 48 hrs

Evaluation : 14 Days

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations:

- The Animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hrs.
- Special attention: First 1-4 hrs after administration of drug, and
- It is observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0-hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

C. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the Animals used, number of Animals displaying signs of toxicity, the number Animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing ***Soodhaga Mezhugu*** with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle [85]

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique.
Results were noted and tabulated in Table. No:17, 18, 19, 20, 21.

4.3.2. REPEATED DOSE 28-DAY ORAL TOXICITY (407) STUDY OF *SOODHAGA MEZHUGU* [86]

IAEC approved Number	**:**01/321/PO/Re/S/01/CPCSEA dated 12/10/2018.
Test Substance	: ***SOODHAGA MEZHUGU***
Animal Source	**:** The animal house of Kings Institute, Guindy, Chennai.
Animals	: Wistar Albino Rats (Male -5, and Female-5)
Age	**:** 6-8 weeks
Body Weight	**:** 150-300gm.
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid
Diet	**:** Pellet feed supplied by Sai Meera foods Pvt Ltd, Bangalore.
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** Between 22ºC $\pm$ 3ºC.
Relative humidity	**:** Between 30% and 70%,
Air changes	**:** 10 to 15 per hour
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**: 28 Days.**

Justification for Dose Selection:

The results of acute toxicity studies in albino Rats indicated that ***Soodhaga Mezhugu*** *was* non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between Rats and human, the doses selected for the study were 100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and Administration of Dose:

Soodhaga Mezhugu *at* three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to Rats at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control Rats were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Methodology

Randomization, Numbering and Grouping of Animals:

Ten Rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Rats were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Table.No.6. Grouping of animals

Groups	**No of Rats**
Group I Vehicle control	10 (5male,5female)
Group II *SM*- low dose 100mg/kg	10 (5male,5 female)
Group III *SM* - Mid dose 200mg/kg	10 (5male,5female)
Group IV *SM* - High dose 400mg/kg	10(5male,5female)

SM – SOODHAGA MEZHUGU

OBSERVATIONS

Experimental Rats were kept under observation throughout the course of study for the following:

Body Weight*:* Weight of each Rats was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs: All Rats were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality*:* All Rats were observed twice daily for mortality during entire course of study.

Functional Observations: At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations: Following laboratory investigations were carried out on day 29 in Rats fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

On 29th day, the Rats were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of hematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Hematological Investigations: Blood samples of control and experimental Rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

A Biochemical Investigations: Serum was used for the estimation of biochemical parameters. Samples of control and experimental Rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy*:* All the Rats were sacrificed on day 29. Necropsy of all Rats was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology: Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto Technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were stained with Hematoxylin-eosin. The organs included kidneys, liver, spleen of the Rats were preserved they were subjected to histopathological examination. Results were noted and in Figure. No:19, 20.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnett's multicomparision test using a computer software programme GRAPH PAD 8 version. Results were noted and tabulated in Table. No: 22, 23, 24, 25, 26, 27, 28.

4.4. PHARMACOLOGICAL STUDY [87]

Ovulation inducing activity in female Wister albino rat model

Animal selection and maintenance

Species	-	Rat
Strain	-	Wistar albino
Sex	-	Female
Age/weight at start of test	-	2 months / 88-130 g
Acclimatization Period	-	7 days prior to dosing
Housing	-	Individually in polypropylene cages
Husbandry	-	12-h light/12-h dark artificial photoperiod / Room temperature 22°C (±3°) and relative humidity 30–70%
Feed and Water	-	Rodent pellet feed (Sai Meera) purified water *ad libitum*
Identification	-	Animals were kept in individual cage and numbered.

Estimation of hormone level:

Method

Before starting drug treatment, the reproductive cycles of the rats were synchronized by the following method. 100μg estradiol dissolved in 2 ml olive oil was injected subcutaneously. All rats after a 24 hr period, received intramuscular injections of 50 μg progesterone dissolved in olive oil. After few hours, vaginal smears were obtained by vaginal lavage to monitor ovulation and estrous cycle. Vaginal smears were prepared by washing vaginal opening with 0.9% w/v of sodium chloride with a glass dropper and placed in a clean glass slide and viewed under light microscope at 40X magnification. Examination of vaginal smears showed that all the animals were in the estrous stage. (Mar codes FK et al).

All the animals were weighed daily after drug administration for 10 days. The suitable sensitive rats were divided into four groups of six each as follows.

Experimental design

- Group I Normal Control animals given only 2ml/kg of CMC solution.
- Group II rats were administered 200mg/kg of ***Soodhaga Mezhugu*** *for* 10days,
- Group III rats were received 400mg /kg of ***Soodhaga Mezhugu*** *for* 10 days
- Group IV received Clomiphene 10mg/kg and served as standard. All the drugs were given orally.

After that 2ml of blood was collected by retro orbital puncture. Blood samples were centrifuged for 15 minutes at 4000 rpm and the separated serum samples were frozen at -20°C and kept for later estimation of LH, FSH and Estradiol by ELISA method. Results were noted and tabulated in Table. No:29 & Chart. No:1.

4.4.2. Hormonal assay

Biochemical assay

The method employed was Microwell Enzyme Linked Immunosorbent Assay (ELISA) using analytical grade reagents.

Estimation of serum luteinizing hormone (LH)

The method employed was Microwell immunoassay (ELISA) using analytical grade reagents. 0.050ml of the serum was pipetted into the assigned wells. 0.001ml of LH-Enzyme reagent was added to all the wells. The microplate was swirled for 20-30 seconds and covered; this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Estimation of serum follicle stimulating hormone (FSH)

The method employed was Microwell immunoassay (ELISA) using analytical grade reagents. 0.050ml of the serum was pipetted into the assigned wells. 0.001ml of FSH-Enzyme reagent was added to all the wells. The microplate was swirled for 20-30 seconds and covered; this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins.The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Determination of serum progesterone levels

The method employed was Microwell immunoassay (ELISA) using analytical grade reagents. 0.025ml of the serum was pipetted into the assigned wells. 0.050ml of progesterone Enzyme reagent was added to all the wells. The microplate was swirled for 20 seconds to mix, 0.050ml progesterone biotin reagent was added to all the wells, the mixture was swirled for 20 seconds to mix and covered, this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for twenty minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Determination of serum Estradiol levels

The method employed was Microwell immunoassay (ELISA) using analytical grade reagents. 0.025ml of the serum reference was pipetted into the assigned wells.

0.050ml of Estradiol Biotin reagent was added to all the wells. The microplate was swirled for 20 seconds to mix, the mixture was incubated at room temperature for 30mins,0.050ml Estradiol enzyme reagent was added to all the wells, the mixture was swirled for 20 seconds to mix and covered, this mixture was allowed to incubate for 90 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for twenty minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a microplate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve

Statistical Analysis

Follicle stimulating hormone, luteinizing hormone, progesterone and estradiol measurements are presented as mean ± SEM. One-way Analysis of Variance (ANOVA) was used to analyses the data ($P<0.05$). Results were noted and tabulated in Chart. No:2.

4.4.3 ANTI OXIDANT ACTIVITY [88]:

DPPH RADICAL SCAVENGING ASSAY [89]

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

PROCEDURE

Different volumes of extracts 1.25µl - 20µl (12.5 - 200µg/ml) from a stock concentration 10mg/ml were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

CALCULATION

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

Results were noted and tabulated in Table. No:31 & Chart. No: 3.

5. RESULTS AND DISCUSSION

In this present thesis *Soodhagavayu* compared to PCOS based on *Siddha* literature. One of the *Siddha* herbo-mineral formulations, *Soodhaga Mezhugu* mentioned for *Soodhagavayu* in *Siddha* literature. The trial drug *SM* indicated for PCOS under the scientific validation through toxicity studies, physicochemical, biochemical and pharmacological activity are done to justify the ovulation inducing activity of *SM*, to evaluate the level of FSH, LH, Progesterone, Estradiol and Anti-oxidant activity. Literary collections about the drug from various text books give hope about its activity. The studies strongly supported the fact through these results.

They are discussed below:

The extensive review on botanical aspect gave information about the Microscopical, Macroscopical, medicinal uses, constituents and the importance of the herbs in detail. Most of the herbs included in the formulation are Anti-bacterial, Anti-microbial, Anti pyretic and Tonic**.**

Discussion on Gunapadam review [(90)]:

The *Karasaram* of *Venkaram* is very important drug for preparing *parpam, chendooram* and *guru.* It has an Emmenagogue action and astringent taste. It is useful in menstrual related problems.

Valendrapolam has a stimulant and Emmenagogue action and bitter taste which is helpful in the hormone related problems.

Kunkumapoo has a stimulant, anodyne and Emmenagogue action and bitter taste. It's also helpful in menstrual and hormone related problems.

Lavangam has a tonic and stimulant action. It is useful in menstrual irregularities.

Discussion on modern drug review:

Borax is an important, and often underutilized, trace mineral naturally present in certain foods and also within the environment. As a mineral that can help balance levels of sex hormones in women including estrogen. Boron helps relieve menopause symptoms as well as PMS, and also for increasing fertility. In animal studies, boron

depletion is linked with fertility problems and birth defects, which suggests that boron can play a role in healthy reproduction and fetus development [(91)].

The disease-causing inflammation, Myrrh also appears to support the body in reducing disease causing oxidative stress. The antioxidant potential of myrrh is so high that it may support healthy liver function. The production of various substances necessary for the pituitary gland to release hormones [(92)].

The effects of saffron *Crocus sativus* on blood level of follicle-stimulating hormone (FSH), and number and dynamics of body weight of offspring in female rats [(93)].

The antioxidant activity of saffron could be attributed to two bioactive compounds, crocin and saffranal, and a DPPH radical scavenging test on crocin and saffranal exhibited antioxidant activities of 65% and 34%, respectively, at 500 ppm.

Clove (*Syzygium aromaticum*) is a rich source of bioactive compounds. The goal of this study was to test different extracts of clove in terms of their phenolic contents, their antioxidant potential and their antibacterial action against pathogenic bacteria[(94)].

Discussion of pharmacological review:

The pharmacological aspect of the review shows the presence of various animal models available for PCOS.

The current pharmacological method available for carrying out the ovulation inducing activities were explained clearly and the suitable animals for carrying out the activities were discussed to be Wistar albino rats than other animals because of the parallelism with the human.

They are also more sensitive when compared to other animals and hence they were chosen for the study.

The exact ovulogenic activity could never be better studied by analyzing the pharmacological activities like Ovulation inducing activity, Antioxidant activity and estimation of hormone level in Female Wistar Albino rats.

Since the three activities could clearly explain whether the drug effectively induces ovulation and maintains the level of hormones which gives the knowledge about the activities.

Discussion on pharmaceutical review

This review explained the preparation of *SM* in detail including the purification of raw drugs, methods of manufacturing *Mezhugu* and the *Siddha* parameters for the standardization of *Mezhugu* analyzing.

Mezhugu:

- 5 years of shelf life denotes its long-time efficacy.
- Being very fine Micro particles, this micro particle size range helps to attain the bio-availability of the drug ***SM*** and aid in reaching the target site of action [(95)].

Discussion on materials and methods

The selection of trial drug was taken from the **"*Kosayee Anuboga Vaithiya Biramma Ragasiyum*"** written by ***Munusamy mudaliyar.***

The ingredients were bought from the authenticated vender and they were identified and authenticated by the experts in Post Graduate Department *Gunapadam,* GSMC, and Chennai.

The SOP of trial drug was done at the well-equipped lab of the Post Graduate Department of *Gunapadam.* So, the principles of GMP were adhered during process.

The herbo-mineral formulation *Soodhaga Mezhugu* had been subjected to various studies for its scientific validation and safety assessment. Literary collections, physicochemical, biochemical and heavy metal analysis, Toxicological study, Pharmacological studies are done to prove its efficacy.

Organoleptic characters:

Table No.7. Organoleptic characters of *Soodhaga Mezhugu*:

S.No	Parameter	Result
1.	Colour in day light	Yellowish Brown
2.	Odour	Pleasant odour
3.	Sense of touch	Soft
4.	Appearance	Semisolid
5.	Taste	Bitter & Astringent
6.	Solubility	Soluble in water and alcohol

Interpretation:

The physical parameters like color, odor, touch, appearance, taste revealed that *Soodhaga Mezhugu* is a Yellowish brown in Color, Soft, Pleasant odor, Bitter & Astringent in taste.

Table.No.8. Results of Physicochemical Parameters

S.No	Parameter	Percentage
1.	Specific gravity	0.929
2.	pH	9.07
3.	Particle size	Completely passes through sieve no. 125.
4.	Loss on drying at 105 degree Celsius	11.32%
5.	Total ash value	42.71%
6.	Acid insoluble ash	3.5%
7.	Water soluble ash	37.67%
8.	Water soluble extraction	23.2%
9.	Alcohol soluble extraction	8%

Discussion on Physicochemical parameters:

Solubility:

- Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for desired (anticipated) pharmacological response.
- Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration; oral bioavailability depends on solubility [(96)].

Discussion:

SM is soluble in major solvents and sparingly soluble in water, well soluble in Hcl, H_2SO_4. *SM* proves that its efficiency of solubility increasing the bio availability.

Specific gravity:

The trial drug *Soodhaga Mezhugu* shows low specific gravity (0.929) compared to water. This indicates its good nature of absorption of drug.

pH value

The pH of *SM* is 9.07. It is alkaline in nature. Our body functions are best when the pH is alkaline. Alkaline pH keeps our body tissue supple and reduces inflammation.

In our body, the end product of all metabolisms is acids. If our drug also in acidic pH causes disturbed homeostasis mechanism.

The advantages of alkaline pH [97]

- Decrease aging process
- Alkaline medium enhances the mineral storage in order to buffer
- Increase utilization of oxygen level in body, which reduces fatigue, carry out cell repair, enhances ability of mitochondria function
- Also controls infection, inflammation of organs reduces microorganism toxicity (Decrease proliferation of microorganism).

Loss on drying

Loss on drying value for *SM* – 11.32%

- Loss on drying (LOD) gives the total amount of volatile content and moisture (water) present in the drug.
- The stability of a drug and its shelf-life are dependent on moisture content.
- Being a *Mezhugu*, the volatile substances are slightly high in *SM*.

Ash values

Total Ash value for *SM* – 42.71%

- Ash values are helpful in determining the quality and purity of the drugs
- It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration [98].
- The total ash value of *Soodhaga Mezhugu* is 42.71%, which determines the presence of inorganic content.

Acid insoluble ash value

- Acid insoluble ash value for *SM* – 3.5%
- The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluble ash value of *Soodhaga Mezhugu is* 3.5%.

Water soluble ash value

Water soluble ash value for *SM* – 37.67%

- Water soluble ash is a part of total ash value, which denotes the diffusion capacity of the drug.
- Here, the water-soluble ash value of *SM* is 37.67%, which represents easy facilitation of diffusion and osmosis mechanism.

Water-soluble and Alcohol-soluble extraction

- Water-soluble extractive values of ingredients and formulation of ***Soodhaga Mezhugu*** are depicted in table which shows 23.2%.

- Higher water-soluble extractive value implies that water is a better solvent of extraction for the formulation than ethanol.

- Alcohol-soluble extractive values of ingredients and formulation of ***Soodhaga Mezhugu*** are depicted in table which shows 8%.

- The results of Alcoholic and Water-soluble extracts of the formulation show that alkaloids of the formulations are more soluble in water than alcohol and a higher water-soluble extractive value of the formulation depicts that water is a better solvent of extraction for the formulation than alcohol.

Phytochemical analysis:

The phytochemical analysis of *Soodhaga Mezhugu* result were given below:

The preliminary phytochemical studies of aqueous extract of ***Soodhaga Mezhugu*** were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of ***Soodhaga Mezhugu.***

Table.No:9. Phytochemicals screening test of *Soodhaga Mezhugu*

S.No.	Phytochemicals	Test Name	H_2O Extract
1.	Glycoside	Modified Borntrager's Test	+ve
2.	Saponin	Foam Test	+ve
3.	Proteins	Xanthoprotein Test	+ve
4.	Amino acids	Ninhydrin Test	+ve
5.	Diterpenes	Copper Acetate Test	+ve
6.	Gum & Mucilage	Extract + Alcohol	+ve

+ve/-ve present or absent if component tested

The above stated phytochemical properties for the given sample certified to be present.

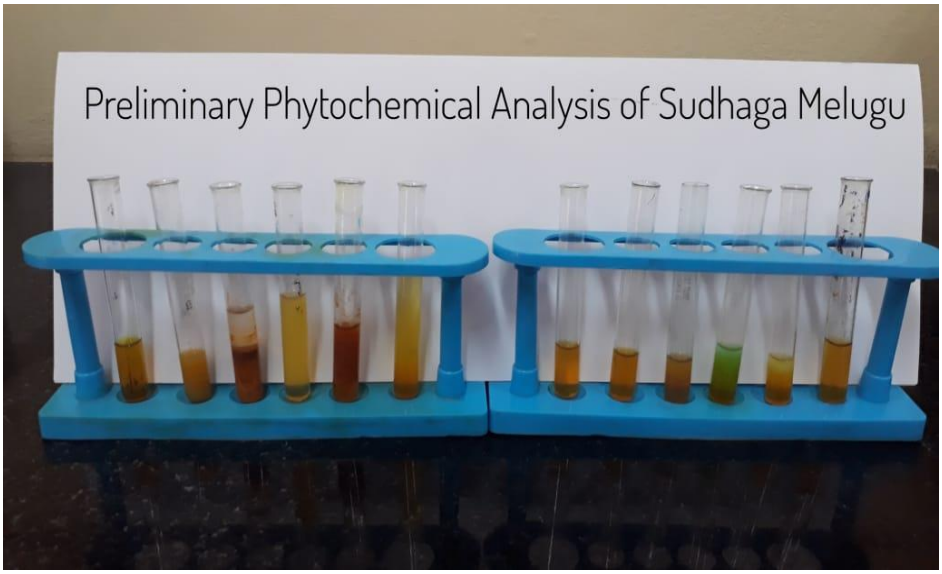


Fig.No.16. Preliminary phytochemical analysis of *Soodhaga Mezhugu.*

The Phytochemical analysis of the drug (*SM)* result reveals Glycoside, Saponin, Proteins, Amino acids, Diterpenes, Gum & Mucilage. The interpretation of the result was given below.

Glycosides:

- Glycosides have antibacterial activity, so they protect our body from bacteria and infectious diseases.
- Glycosides increased the intestinal motility. So, it produces laxation [(99)].
- A glycoside is a molecule consisting of a sugar and a non-sugar molecule, called an aglycone. Many biologically active compounds are glycosides.
- The pharmacological effects are largely determined by the structure of the aglycone [(100)].

Saponins:

- In the digestive tract, saponins produce an emulsification of fat-soluble molecules.
- Saponins bind with bile acids and helps to eliminate them from the body, preventing cholesterol from being reabsorbed.
- Saponins can boost the immune system, have an antioxidant effect and may even support bone strength [(101)].

Proteins and Amino acids:

- Lifestyle contributors to disease include not only calorie excess but also the dietary intake of specific nutrients. Advanced glycated end-products (AGEs) is a class of nutrients incriminated in the pathogenesis of diet-related diseases. Cooking or processing at high temperatures such as broiling, grilling, frying and roasting is the major source of AGEs. The AGEs promote oxidative stress and insulin resistance in peripheral tissues. PCOS women have increased serum AGEs levels, and these have been positively correlated with serum androgen levels. By activating protein kinase C, AGEs may impair insulin action, thereby perpetuating insulin resistance in PCOS.

Diterpenes:

- Diterpene has an anti-oxidant effect.
- Diterpenes helps to cure hypertension. It also has tumour inhibitory properties as well as a stimulating effect on the immune system.
- It is used widely as a stomachic [(102)].

Gum & Mucilage:

- It is used as a bulk laxative.
- Gum and mucilage are used for their demulcent properties for cough Suppression [103].

Bio-Chemical analysis

Table. No. 10. Results of basic radical studies

S. No	Parameter	Result
1.	Test for Potassium	Positive
2.	Test for Iron (Ferrous)	Positive
3.	Test for Zinc	Positive
4.	Test for Aluminum	Positive

Table.No.11. Test for acid radical studies

S.No	Parameter	Result
1	Test for Sulphate	Positive

Discussion on Biochemical analysis

The Biochemical analysis for basic radicals of *SM* shows the presence of Magnesium, Iron.

Potassium

- Potassium is very important in cellular biochemical reactions and energy metabolism. It participates in the synthesis of protein from amino acids in the cell.
- Potassium also functions in carbohydrate metabolism. It is active in glycogen and glucose metabolism, converting glucose to glycogen that can be stored in the liver for future energy.
- Potassium is important for normal growth and for building muscle [104].

Iron:

- Iron is used to maintain healthy cells, skin, hair and nails.
- Iron is an essential element for blood production and also production of enzymes, hormones, amino acids and neurotransmitter.
- Insufficient amount of iron affects the ovulation.
- Iron separately implicated in the development or protection against disease.
- Health benefits of iron also include the elimination of unexplained or chronic fatigue, which may occur in both men and women. Its deficiency is a natural cause of fatigue since it is an important component of hemoglobin [(105)].

Zinc

- Zinc is a very important element in the reproductive cycle of species. In humans, it is necessary for the formation and maturation of spermatozoa, for ovulation and for fertilization.
- During pregnancy, zinc deficiency causes a number of anomalies, spontaneous abortion, pregnancy-related toxemia, extended pregnancy or prematurity, malformations and related growth. Delivery is adversely affected by deficiency.
- These different effects of zinc can be explained by its multiple action on the metabolism of androgen hormones, estrogen and progesterone, together with the prostaglandins. Nuclear receptors for steroids are all zinc finger proteins.
- Zinc supplementation has already proven beneficial in male sterility and in reducing complications during pregnancy. However, it would be worth conducting larger-scale trials to confirm these beneficial effects [(106)].
- Zinc also helps the pituitary gland to release follicle-stimulating hormone (FSH). FSH encourages ovulation and tells your ovaries to produce more progesterone [(107)].

Aluminium

- Aluminum is a great source for various products and compounds that actually improve health.
- Aluminum is the third most common element in the earth crust after oxygen and silicon. Humans have, in other words, evolved and lived in an aluminum-rich environment since the beginning of man. This also means that we're well

adapted to this element and we're exposed to aluminum in many ways, every day [108].

The biochemical analysis for acidic radicals of *SM* shows the presence of Sulphate.

Sulphate

- Sulphate containing proteins work in indirect ways to maintain the hormone level. So, it is useful in menstrual irregularities [109].

The acid basic radical results show presence of Potassium, Zinc, Aluminum, Iron and Sulphate which increase the therapeutic effect of *SM*.

HPTLC (High Performance Thin Layer Chromatography)

Sample Name **: *Soodhaga Mezhgu***

Stationary Phase : Silica Gel 60 F_{254}

Mobile Phase – Toluene : Ethyl acetate: Acetic acid (5: 1.5: 0.15 v/v/v)

Results of HPTLC study:

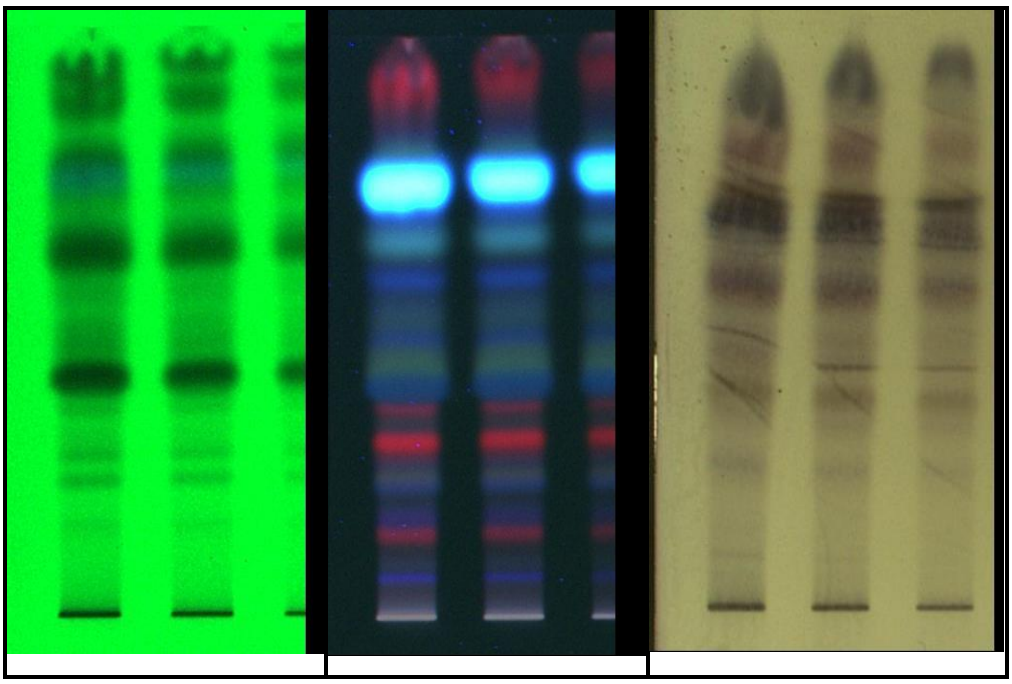
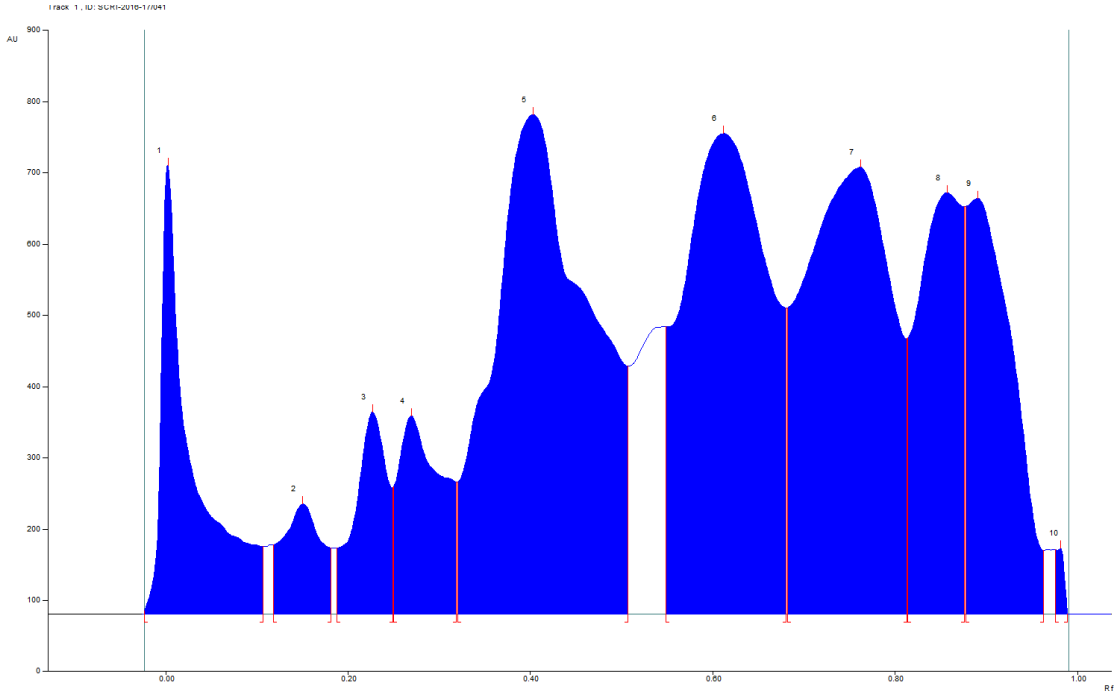


Table.No.12. HPTLC finger print

λ = 254 nm		**λ = 366 nm**		**λ = 575 nm (Derivatized)**	
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Grey	0.14	Blue	0.07	Blue	0.24
Grey	0.22	Red	0.14	Brown	0.36
Grey	0.27	violet	0.18	Brown	0.39
Grey	0.34	Blue	0.22	Yello W	0.51
Bright Blue	0.40	yellow	0.25	Brown	0.52
Grey	0.53	Red	0.29	Black	0.65
Black	0.60	Red	0.35	Violet	0.75
Grey	0.70	Blue	0.38	Black	0.89
Blue	0.72	Green	0.44		
Black	0.77	Blue	0.46		
Black	0.85	Blue	0.57		
Black	0.92	Light blue	0.62		
		Bright blue	0.72		
		Red	0.88		

Table.No.12a. Results of HPTLC study

HPTLC Chromatogram @ 254 nm:



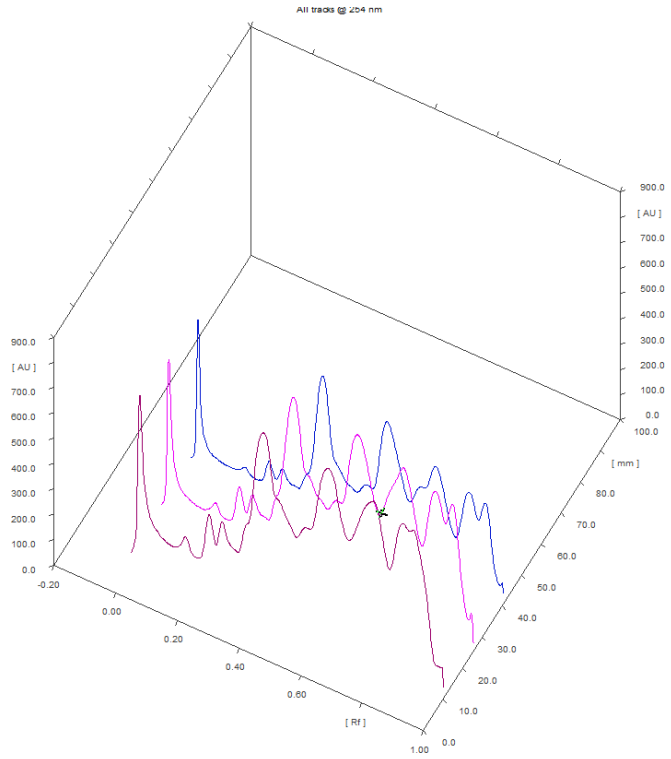
Graph. No.1. HPTLC Chromatogram @ 254 nm

Peak Table @ 254 nm:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	4.1 AU	0.00 Rf	629.4 AU	13.63 %	0.11 Rf	95.1 AU	18982.5 AU	6.89 %
2	0.12 Rf	97.2 AU	0.15 Rf	154.6 AU	3.35 %	0.18 Rf	93.0 AU	5971.7 AU	2.17 %
3	0.19 Rf	92.9 AU	0.23 Rf	284.0 AU	6.15 %	0.25 Rf	77.5 AU	8934.8 AU	3.24 %
4	0.25 Rf	179.2 AU	0.27 Rf	278.2 AU	6.03 %	0.32 Rf	85.4 AU	11844.2 AU	4.30 %
5	0.32 Rf	185.6 AU	0.40 Rf	701.2 AU	15.19 %	0.51 Rf	47.7 AU	66460.6 AU	24.13 %
6	0.55 Rf	403.4 AU	0.61 Rf	674.7 AU	14.61 %	0.68 Rf	29.2 AU	56459.6 AU	20.50 %
7	0.68 Rf	429.4 AU	0.76 Rf	627.1 AU	13.58 %	0.81 Rf	86.4 AU	53919.7 AU	19.58 %
8	0.82 Rf	387.6 AU	0.86 Rf	591.4 AU	12.81 %	0.88 Rf	71.6 AU	25830.9 AU	9.38 %
9	0.88 Rf	572.1 AU	0.89 Rf	583.7 AU	12.64 %	0.96 Rf	88.9 AU	26289.8 AU	9.54 %
10	0.98 Rf	90.1 AU	0.98 Rf	92.9 AU	2.01 %	0.99 Rf	-0.0 AU	750.5 AU	0.27 %

Table. No.13. Peak Table @ 254 nm

3D Chromatogram @ 254 nm:



Graph. No. 2. 3D Chromatogram @ 254 nm

A qualitative finger printing of *Soodhaga mezhugu* has been performed by HPTLC method, which provides qualitative insights into the bioactive constituents present in the drug. HPTLC shows separation of components present in the chloroform extract of *Soodhaga mezhugu.*

The present study revealed that *Soodhaga mezhugu* showed best results in Toluene: Ethyl Acetate: Acetic Acid 5: 1.5: 0.15 solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366nm and 575nm and visible light range.

TLC plate showed different colour phytoconstituents of chloroform extract of *Soodhaga mezhugu.* The bands revealed presence of bright blue, greenish blue, violet and dark pink, showing the presence of steroids, terpenoids, alkaloids, flavonoids, tannins, lignans and saponins.

The results from HPTLC finger print scanned at wavelength 366nm for chloroform extract of *Soodhaga mezhugu.* There are eleven polyvalent

phytoconstituents and corresponding ascending order of R_f values start 0.07 to 0.88 in which highest concentrations of the phytoconstituents was found to be 15.19% and 14.61% with its corresponding R_f value were found to be 0.32 and 0.55 respectively.

ANTI-MICROBIAL ACTIVITY

GRAM NEGATIVE

Table.No.14.1. Organism: *E. coli*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Soodhaga Mezhugu (SM)	Streptomycin (100µg)	26
	250	15
	500	17
	1000	20

14mm-Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

Table.No.14.2. Organism: *Klebsiella pneumoniae*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Soodhaga Mezhugu (SM)	Streptomycin (100µg)	25
	250	17
	500	19
	1000	23

14mm-Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

Table.No.14.3. Organism: *Pseudomonas aeroginosa*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Soodhaga Mezhugu (SM)	Streptomycin (100µg)	30
	250	15
	500	19
	1000	24

14mm-Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

GRAM POSITIVE

Table.No.14.4. Organism: *Staphylococcus aureus*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Soodhaga Mezhugu (SM)	Streptomycin (100µg)	26
	250	16
	500	18
	1000	21

14mm-Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

ANTI-FUNGAL ACTIVITY

Table.No.14.5. Organism: *Aspergillus niger*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Soodhaga Mezhugu (SM)	Clotrimazole(100µg)	37
	250	17
	500	19
	1000	25

14mm-Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

Inference:

1. *Escherchia.coli* - Highly sensitive in 500(µg/mL)
2. *Klebsiella pneumoniae* - Highly sensitive in 250 (µg/mL)
3. *Pseudomonas aeruginosa* - Highly sensitive in 500(µg/mL)
4. *Staphylococcus aureus* - Highly sensitive in 250 (µg/mL)
5. *Aspergillus niger* - Highly sensitive in 250 (µg/mL)

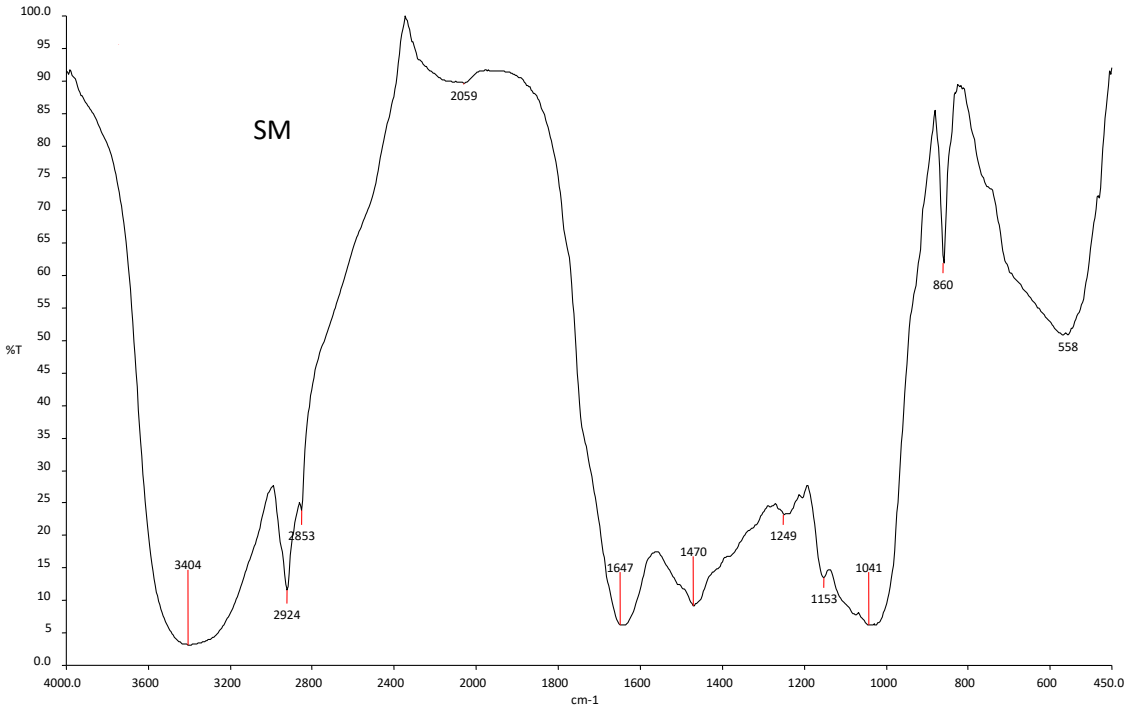
Discussion:

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial and anti-fungal agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *SM* was compared with Standard drug Streptomycin and Clotrimazole (100µg)/ml disc for the following pathogens, they are *Escherchia coli*,

Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Aspergillus Niger. The results represent *SM* potentially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The findings reveal that the *Siddha* drug *SM have* anti-microbial potency against bacterial and fungal pathogens which is used in the treatment of diseases.

SOPHISTICATED INSTRUMENTAL ANALYSIS:

FTIR -FOURIER TRANSFORM INFRARED SPECTROSCOPY



Graph. No. 3. FTIR -FOURIER TRANSFORM INFRARED SPECTROSCOPY

Table.No.15. FTIR Interpretation of *Soodhaga mezhugu*

Absorption peak cm-1	**Stretch**	**Functional Group**
3404	O-H stretch, H-bonded	Alcohols, Phenols
2924	C-H stretch	Alkanes

2853	C-H stretch	Alkanes
1647	N-H bend	1^0 amines
1470	C-H bend	Alkanes
1249	C-O stretch	Alcohols, carboxylic acids, esters, ethers.
1153	C-H wag ($-CH_2X$)	Alkyl halides
1041	C-N stretch	Aliphatic amines
860	C-Cl stretch	Alkyl halides
558	C-Br stretch	Alkyl halides

Functional groups are structural units within the organic compound that are defined by specific bonding arrangements between specific atoms and it means a group of atoms the shape of which determines the characteristic chemical properties of the molecule.

The wave numbers from 4000cm-1 to 1500cm-1 gives details for identification of functional group.

The wave number from 1500cm -1 to 400 cm-1 provides particulars about molecular fingerprint.

The FT IR spectra analysis which exhibits the 9 peak value 3404, 2924, 2853, 1647, 1470, 1249, 1153, 1041, 860, 558cm having O-H stretch, H-bonded, C-H stretch, N-H bend, C-H bend, C-O stretch, C-H wag($-CH_2X$), C-N stretch, C-CI stretch, C-Br stretch.

This peak indicates the presence of functional groups such as Alcohol, Phenols, 1^0 Amines, Alkanes, carboxylic acids, esters and ethers, Aliphatic amines, Alkyl halides.

Alcohol

- The study published in the British Medical Journal (BMJ), suggested that drinking in moderation could offer a protective effect for the heart compared with not drinking.
- Alcohol in moderation has a reputation for being healthy for the heart. Drinking about a glass of wine for women per day, and two glasses for men, in linked to a lower risk of heart attack, stroke and death from heart disease. It has anti-microbial action and acts as an antiseptic agent.
- Alcohol controlling the inflammation and analgesic effect.

OH

OH, group of *SM* has higher potential towards inhibitory activity against microorganisms.

Phenols

- Phenolic compounds are also present in a number of biological systems and natural products such as neurotransmitters, flavoring agents, and vitamins to name a few.
- The effect of phenols is currently of great awareness due to their anti-oxidative. Phenols and flavonoids possess diverse biological activities, for example antioxidant and antidepressant activities [(110)].
- The abnormal oxidative stress in polycystic ovary syndrome (PCOS) could cause genetic instability and raise the risk of cancers. Antioxidants have positive effects on PCOS.

Amines:

- Amines are inorganic derivatives of ammonia; they play a very significant role in the creating amino acids.
- Amine groups act on the neurotransmitters; and also, it is involved in the protein synthesis.
- Proteins are helpful in creation of hormone and enzyme [(111)].

Alkanes

Alkanes are said to be saturated hydrocarbons, because the carbons are bonded to the maximum possible number of hydrogen's - in other words, they are *saturated* with hydrogen atoms [(112)].

Carboxylic acid [113]

- Carboxylic acids make up a series of fatty acids that are extremely good for health. The omega-6 and omega-3 fatty acids are considered to be "essential" because they are required for good health yet cannot be produced by the body.
- Another name for omega-6 is linoleic acid. It helps to maintain cell membranes and to control nutrient use and metabolism and also regulating the hormone level.
- Omega 3 fatty acids increase insulin sensitivity, reduce hyperinsulinemia, lower plasma triglyceride and liver fat, decrease inflammation and obesity.

Alkyl halides

Alkyl halides are also known as haloalkanes. It is required for the synthesis of glutathione and anti-oxidant activity.

ICP-OES (INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY):

ICP-OES is an analytical technique used for the detection of trace metals. The intensity of this emission is indicative of the concentration of the elements within the drug *SM* (wt:0.20350 mg).

Table.No:16. ICP-OES Interpretation of *Soodhaga mezhugu*

S.No	Elements	Wavelength(nm)	Concentration
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	21.110mg/L
4.	Cd	228.802	BDL
5.	Cu	327.393	BDL
6.	Fe	238.204	01.054
7.	Hg	253.652	BDL
8.	Mg	285.213	01.224mg/L
9.	Na	589.592	04.321mg/L
10.	Ni	231.604	BDL
11.	Pb	220.353	BDL
12.	P	213.617	58.117mg/L

BDL- Below Detecting Limit:

1% = 10000 ppm, (1 ppm = 1/1000000 (or) 1 ppm = 0.0001%)

The toxic metals and the permissible limits

Heavy metals	**WHO & FDA limits**
Arsenic (As)	10ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

Interpretation:

Calcium and phosphorus

Calcium (Ca) plays an important role in gonadotropic regulation of ovarian steroidogenesis. Marginal deficiency of phosphorus cause disturbance in the pituitary-ovarian-axis including ovulation. It also suggests that Ca is involved in the distribution of cumulus cell cohesiveness by regulating the number of gap junctions between the cells, which contributes to the process of ovulation [(114)].

Iron

- Iron improves the hematological level in the body and also it is essential for growth, reproduction, healing and immune function.
- In this, iron is present in ferrous form, which is soluble and readily absorbed in the intestinal lumen.

Magnesium:

- Magnesium activates over 300 enzyme reactions in the body.
- Magnesium which helps in the hormone creation, it's a great help to our body.
- Magnesium prevent the excess creation of stress hormone cortisol because of excess Cortisol level leads to alteration of progesterone, estrogen, testosterone,

FSH and LH will be too, this is the vital when it comes to healing hormonal issue like PCOS.

- It helps to maintain nerve and muscle function, supports a healthy immune system.
- It also helps regulate blood glucose levels because it enhances insulin secretion and aid in the production of energy and protein and facilitates sugar metabolism. This is vital when it comes to healing hormonal issues like PCOS.
- Thyroid support – magnesium helps the production of the thyroid hormone and as an anti-inflammatory, prevents thyroid diseases. It also prevents the chronic urination that can be a sleep interrupter [115].

Sodium

Sodium are indirectly related to reproduction. Deficiency of sodium can affect the normal reproductive physiology by preventing the utilization of protein and energy. So, presence of the minerals enhances the therapeutic effects [116].

DISCUSSION:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

ICP-OES reveals the concentration of many minerals present in the drug. It also has physiologically important minerals like Ca, Fe, Mg, Na & P. In *Soodhaga mezhugu*, the heavy metals like As, Hg, Pb, Cd and trace element like Ni were below detectable level. This reveals the safety of the drug.

SEM (SCANNING ELECTRON MICROSCOPE)

The following image is done by 20000X magnification via 2µm aperture shows maximum depth focused.

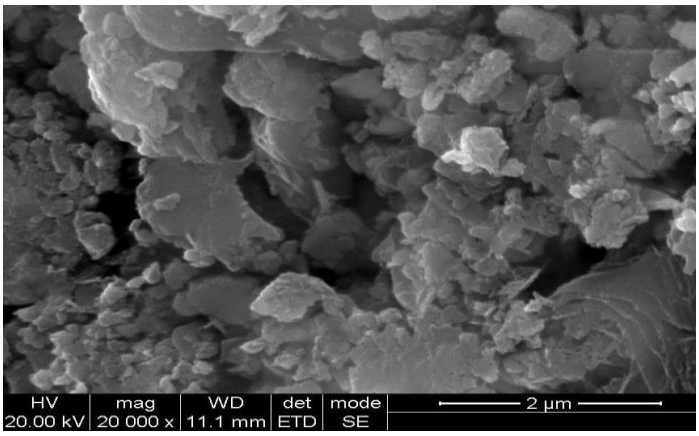


Fig No. 17 Showing micro particles in SEM image of *SM*(2µm) at 20000 x magnification

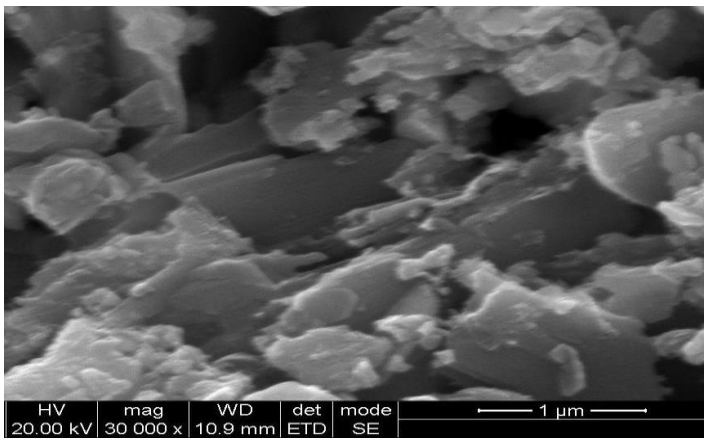


Fig No. 18 Showing micro particles SEM image of *SM*(1µm) at 30000 x magnification

The micro particles present in the drug are of great clinical important. Size and surface micro particles can be easily manipulated to achieve both passive and active targeting.

Advantages of micro particles in drug delivery system:

Biodegradable microparticles have been used frequently as drug delivery vehicles due to its grand bioavailability, better encapsulation, control release and less toxic properties.

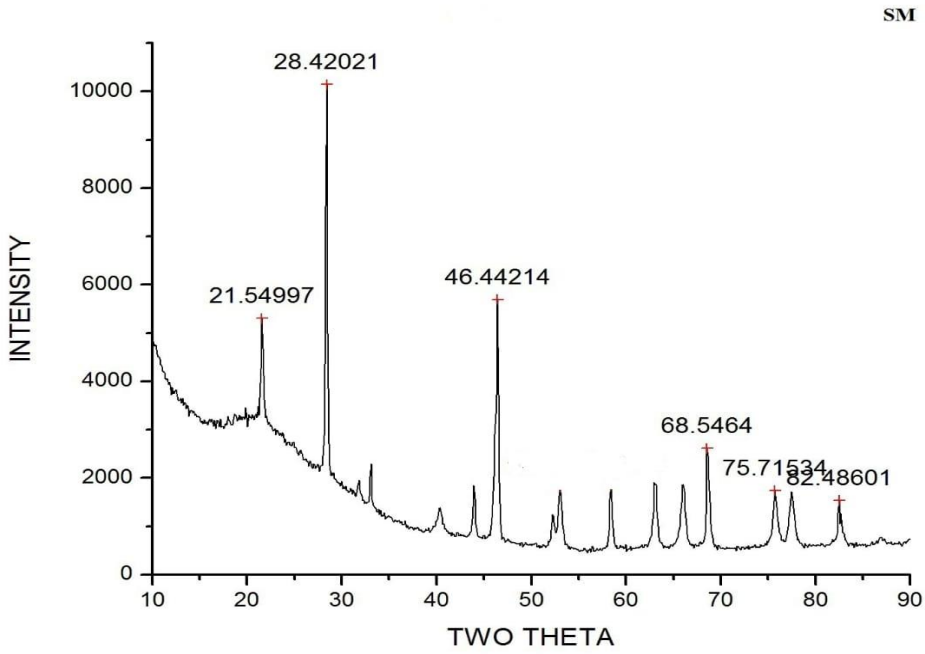
- Increased therapeutic effect of the drug
- Micro particles can be used for directing the drugs to specific target cells
- Decreased toxicity/ side effects
- Protection from physical and chemical degradation
- Improves stability and Increased bioavailability [(117)].

The test drug *Soodhaga Mezhugu* contains Micro particles.

- Micro particles present in the drug results in a better bioavailability and facilitate absorption.
- The particles of micro size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

SEM analysis of the test drug *Soodhaga Mezhugu* revealed the presence of micro particles of size 5-2 Microns. The particles of size micro and near micro size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

XRD (XRAY DIFFRACTION)



Graph.No.4.XRD Interpretation

XRD Interpretation

- The X–ray diffraction pattern of a pure substance is, therefore, like a fingerprint of the substance. It is based on the scattering of X-ray by crystals.

- XRD was used to identify drug substance forms and determine the molecular structure of the sample.
- It well monitoring the crystal morphology of drugs.

Results of XRD:

There are three strongest peaks present in the XRD analysis such as 28.42021, 46.44214 and 21.54997.

Discussion on XRD results:

The crystalline structure, the size and the shape of the particles are highly dependent on the route of synthesis and highlight the therapeutic effect of the drug. The Nano particles may enhance bio absorption of the drug.

Any morphological change in the crystalline state of trail drug as a result of the manufacturing process can influence a drug's bioavailability.

XRD pattern of *Soodhaga Mezhugu* shows the good crystallinity after calcinations process. The major diffraction peaks are identified after XRD analysis *SM* concluded in nano crystalline range (21-46nm) is association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *SM* act as additional supplement and possibly helps in increase the efficacy of the formulation [(118)].

ACUTE ORAL TOXICITY

Dose finding experiment and its behavioral Signs of Toxicity for *Soodhaga Mezhugu.*

Observation done

Table.No.17. Dose finding experiment and its behavioral Signs of acute oral Toxicity

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion	Absence of sign (-)

4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Normal
6	Salivation	Normal	6	Salivation	Normal
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

The animal was observed for 48 hours to watch for twenty parameters, shown in the table below.

Table.No.18. Dose finding experiment and its behavioral Signs of Toxicity of *SOODHAGA MEZHUGU*

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2000mg/kg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

(+ Present, - Absent)

1. *Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7.Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19 Respiration 20. Mortality*

Table.No.19. Body weight Observation

DOSE	DAYS		
	1	7	14
CONTROL	220.2±42.30	322.4 ± 60.10	323.6 ±52.10
HIGH DOSE 400mg/kg/day	262.4± 1.21	282 ± 2.04	284.2 ± 2.10
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table.No.20. Water intake (ml/day) of Wistar albino rats group exposed to *Soodhaga Mezhugu:*

DOSE	DAYS		
	1	7	14
CONTROL	58 ± 1.02	58±9.20	59.4±1.04
HIGH DOSE 400mg/kg/day	59.4±2.20	59.8±3.40	59.9±6.24
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table.No.21. Food intake (gm/day) of Wistar albino rats group exposed to *Soodhaga Mezhugu*

DOSE	DAYS		
	1	7	14
CONTROL	61.04±2.62	62.2±4.76	64.3±6.26
HIGH DOSE 400mg/kg/day	69.4±4.23	70.4±6.22	71.6±4.18
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Acute toxicity Discussion:

- Acute oral Toxicity study was done in Rats according to OECD guideline 423. The drug was administered at the dose of 2000 mg/kg body weight to two groups comprising three Rats in each group.
- It was found that the Rats were alert and responded to touch stimuli, which states that the drug did not interfere with the level of consciousness. The study had shown that the drug did not produce any impact on central nervous system, parasympathetic nervous system, respiratory system.

SUB-ACUTE TOXICITY OF *SOODHAGA MEZHUGU*

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *SOODHAGA MEZHUGU* – (OECD- 407 guidelines)

Soodhaga Mezhugu at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to Rats at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control Rats were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Effect of *Soodhaga Mezhugu* on total body weight in Rats

The total body weight of the Rats was weighed on 1^{st}, 7^{th}, 14^{th}, 21^{st}, 28^{th} day and is shown in the table. It was found that the test drug produced significant weight gain than control, with administration of the drug. Similarly, the test drug at all dose levels induced weight gain and we could see longer the duration of administration of drug higher was the weight gain.

Body weight (g) changes of Rats exposed to *Soodhaga Mezhugu*

Table.No.22. Body weight of wistar albino rats group exposed to *Soodhaga Mezhugu*

Dose (mg/kg/day)	***Days***				
	1	**7**	**14**	**21**	**28**
Control	157.02±2.6	159.11±1.86	160.12±2.20	160.84±2.11	162.14±2.81
100	151.00±1.8	153.25±1.76	149.51±1.86	148.50±1.2	149.50±2.6
200	150.12±2.92	149.12±1.86	153.28±1.96	155.35±2.01	156.38±1.72
400	155.37±1.3	153.37±1.32	153.38±1.02	155.12±1.36	156.38±2.6
P value (p)*	NS	NS	NS	NS	NS

*NS- Not Significant, **(p < 0.01), *(p <0.05), Values are expressed as mean ± S.E.M. N=10*

Table.No.23. Water intake (ml/day) of Wistar albino rats group exposed to *Soodhaga Mezhugu*

DOSE	**DAYS**				
	1	7	14	21	28
CONTROL	60.1 ± 8.72	60±1.52	60.2±1.40	61±1.32	61.4±1.62
LOW DOSE 100mg/kg/day	65.1±1.21	65.6±4.22	65.2±1.02	65.6±2.06	66.4±1.20
MID DOSE 200mg/kg/day	62.1±1.02	62.3±1.21	63.1±4.62	63.4±1.32	63.4±1.64
HIGH DOSE 400mg/kg/day	64.1±1.81	64.2±1.32	64.4±1.14	64.6±1.62	65.8±2.02
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table No.24. Food intake (gm/day) of Wistar albino rats group exposed to *Soodhaga Mezhugu*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	34±4.14	34.2±6.12	34.3±2.18	34.2±1.14	34±5.62
LOW DOSE 100mg/kg/day	36.3±1.64	36.4±1.51	36.2±1.51	36.5±1.22	36.5±1.62
MID DOSE 200mg/kg/day	34.1±2.12	34.2±3.50	34.2±2.14	34.2±2.16	35.2±1.64
HIGH DOSE 400mg/kg/day	32.4±1.62	32.4±1.64	33.6±2.36	34.6±1.20	36.4±2.32
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Effect of *Soodhaga Mezhugu* on Haematological parameters in rats

To evaluate the sub-acute toxicity of ***Soodhaga Mezhugu,*** the haematological parameters like RBC, WBC, platelet, differential count, Hb tests were also executed. The above results showed that all parameters remained within normal limits. The rats did not reveal any observed signs.

Table No.25. Effect of *Soodhaga Mezhugu* on Haematological parameters in rats

Parameter	Control	100mg/kg	200mg/kg	400mg/kg	P value (p)*
RBC (x 10^6/mm^3)	5.2±0.43	5.42±0.46	6.4±0.9	6.16±0.3	N.S
PCV (%)	48.2±1.8	50.2±1.2	51.3±0.9	52.6±0.4*	N.S
Hb (g/dl)	15±0.3	15.8±0.6	16.2±0.4	16.8±0.9	N.S
WBC (mm^3)	8422±183	7780±134	8234±111*	9147±153**	N.S

Neutrophils (%)	18±2	24.2±0.19*	24.6±1.3*	26±2.12.8**	N.S
Mononuclear cells (%)	78±3	72.21±1.56	75.6±0.78	73.2±2.54	N.S
Eosinophils (%)	3±0.3	2.2±0.25*	1.8±0.04**	1.6±0.07**	N.S
Platelets (x 10^3/mm^3)	645±6.2	641±9.2	616±1.5**	672±4.3**	N.S

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=10*

Table No.26. Effect of *Soodhaga Mezhugu* on biochemical parameters in rats

Parameters	**Control**	**100mg/kg**	**200mg/kg**	**400mg/kg**	**P value (p)***
Albumin (g/dl)	2.8±0.2	2.15±0.16	3.14±0.29	4.34±0.18**	N.S
Total Cholesterol (mg/dl)	111.53±13.17	111.6±11.8	103.6±8.6	97.2±5.96	N.S
Triglycerides (mg/dl)	97.56±14.5	96.4±9.8	90.6±6.8	86.4±11.2	N.S
Glucose (mg/dl)	113.4±12.2	104.4±6.8	116.4±11.3	120.6±9.5	N.S
Sodium (mEq/L)	147.3±5.8	148.6±6.9	148.9±4.2	149.3±5.6	N.S
Potassium (mEq/L)	5.3±0.4	4.2±0.13	4.6±0.09	5.9±0.6	N.S

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=10*

The biochemical parameters are in the reference range and this showed non-toxic effects on general body metabolism.

Table.No.27. Renal function test of Wistar albino rats group exposed to *Soodhaga Mezhugu*

Parameters	Control	50mg/kg	200mg/kg	400mg/kg	P value (p)*
Urea (mg/dl)	63±4.3	60.4±3.4	57.6±1.6	67.4±2.4	N.S
Creatinine (mg/dl)	0.53±0.03	0.58±0.04	0.66±0.11	0.72±0.06	N.S
BUN (mg/dl)	21.33±4.6	20.4±2.8	19.6±1.5	28.6±0.98**	N.S

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=10*

Table.No.28. Liver Function Test of Wistar albino rats group exposed to *Soodhaga Mezhugu*

Parameters	Control	50mg/kg	200mg/kg	400mg/kg	P value (p)*
Total Bilirubin (mg/dl)	0.9±0.08	0.64±0.06	0.83±0.02*	0.68±0.09	N.S
SGOT (U/L)	86.5±5.0	98.4±9.8	81.3±6.5	94.2±5.8	N.S
SGPT(U/L)	46.5±6.2	53.6±5.6	48.4±2.9	51.2±4.2	N.S
Alkaline phosphatase (U/L)	48.6±7.2	56.4±5.2	42.3±3.4	48.6±4.6	N.S
Protein (g/dl)	6.33±0.3	6.12±0.5	5.19±0.28	6.26±0.15	N.S

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=10*

Results for repeated 28 days oral toxicity:

Observations

Overall observations were similar in both sex rats.

Clinical signs of toxicity

No clinical signs of toxicity were observed.

Mortality

No mortality was observed after 28 days repeated dose administration of *SM*. All animals survived to study termination period.

Body weight

No significant alterations were observed in body weight.

Food and water consumption

No effect of treatment was noted.

Physiological activities

No changes in the general behavior.

Blood analysis

a. **Hematology**

   No treatment related effects were observed.

b. **Biological parameters**

   No treatment related effects were observed.

c. **Histological examination**

   Histological examination of organs did not show any pathological changes.

Repeated 28 days oral toxicity Discussion:

- The results of acute toxicity studies in albino Rats indicated that *Soodhaga Mezhugu was* non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between Rats and human, the doses selected for the study were 100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.
- No physical changes were observed throughout the dosing period.
- No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.
- No significant changes in Red blood cells (RBC) white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.
- Hence the herbo mineral formulation of *SM* can be considered to be safe drug for prolonged duration use as revealed by toxicological studies.

HISTOPATHOLOGY EXAMINATION

- Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for hematological and blood chemical analyses were taken from common carotid artery.

- All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

Discussion:

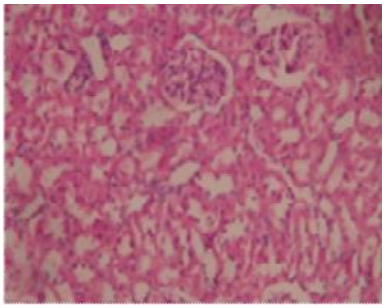
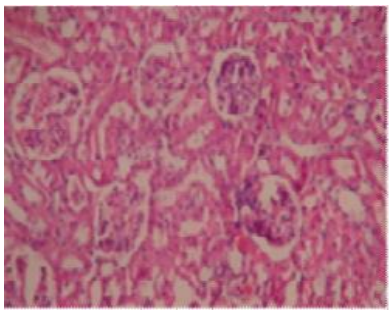
The above slides show the histopathology studies of repeated 28 days oral toxicity studies. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Soodhaga Mezhugu*. Thus, the safety of the drug is revealed, so that it can be administered for long time without any side effects.

HISTOPATHOLOGY SLIDES:

Fig.No.19. HISTOPATHOLOGICAL EVALUATION FOR SUB-ACUTE TOXICITY

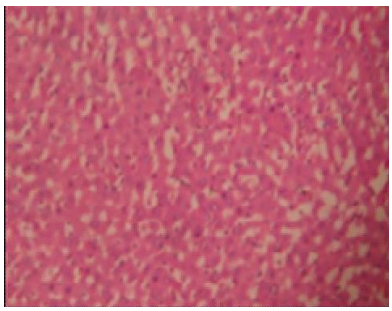
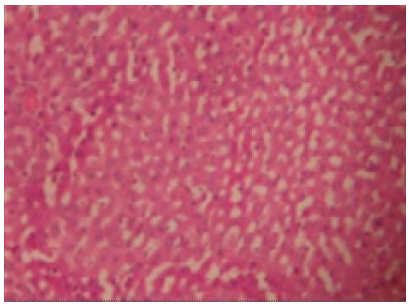
KIDNEY

NORMAL **HIGH DOSE**

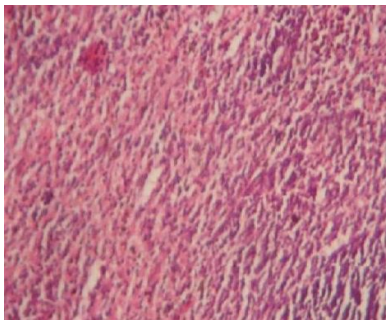
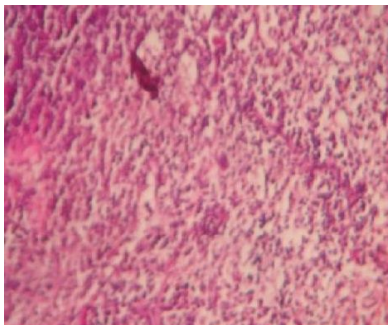
LIVER

NORMAL **HIGH DOSE**

SPLEEN

NORMAL **HIGH DOSE**

PHARMACOLOGICAL STUDY

The *Soodhaga Mezhugu* does not produce any toxic effect on acute and subacute oral toxicity. It was conformed that the *SM* considered being safe at the dose range of 400 mg/ kg.

Ovulation inducing activity:

Ovulation inducing activity was carried out in 6 Wister albino female rats for each group was included in this study. Clomiphene citrate was used as the standard drug, *SM* 200 mg/kg and 400 mg/kg were administrated through enteral route.

Histological analysis:

The ovary was separated from the uterus and placed in formalin fixative for 24 hours. Then these fixed tissue samples were placed in ascending concentrations of alcohol and embedded in paraffin. Tissues were sliced with 3-5μm thickness and stained with hematoxylin and eosin, and then monitored and analyzed with a light microscope. For the evaluation of ovulation inducing activity of trial drug, all tissue blocks were serially sliced. Follicle identification was based on the detection of a nucleus. The numbers of follicles (primordial, primary, etc.) were counted.

Discussion on Ovulation inducing activity:

Histological studies revealed that treatment with doses of 200mg/kg and 400mg/kg of *SM* significantly increased the number of primordial follicles ($p<0.05$ for 200 mg /kg; $p<0.01$ for 400 mg /kg;). This increase was also observed in the number of primary follicles; however, it was significant only in the 200 mg/kg group ($p<0.01$).

And also decreased the number of preantral and antral follicles, 400mg/kg of the *Soodhaga Mezhugu* slightly increased the number of atretic follicles, a greater increase was observed at 200mg/kg. Treatment with doses of 200mg/kg and 400mg/kg of *Soodhaga Mezhugu* significantly increased the number of primary follicles; however, it was statistically significant only in the 200mg/kg group. These were more prominent in rat ovary that received *SM* 200mg and Clomiphene citrate.

SM also caused an increase in the number of atretic follicles, which confirmed the containing effect of natural growth of follicles.

Effect of *Soodhaga Mezhugu* on weight of uterus and ovary

The relative weight of uterus and ovary were significantly increased (P<0.05) in test groups that received *SM* 400mg /kg at the end of the tenth day as compared with CMC treated normal female rats.

Table No.29. Effect of *Soodhaga Mezhugu on* weight of uterus and ovary after 10 days treatment

S.No	Group	Treatment and dose	Weight of uterus (mg)	Weight of ovary (g)
1.	Normal	2ml/kg 2% CMC	16.35±1.2	1.69±0.14
2.	Test-I	SM 200mg /kg	12.12±0.57*	1.48±0.18*
3.	Test-II	SM 400mg/kg	14.27±1.01*	1.54±0.10*
4.	Standard	Clomiphene citrate 10mg/kg	15.48±+0.72	1.70±0.12

*N = 6. Values are expressed as Mean±SEM. *P>0.05 compared to normal control*

Chart. No.1. Effect of *Soodhaga Mezhugu on* weight of uterus and ovary after 10 days treatment

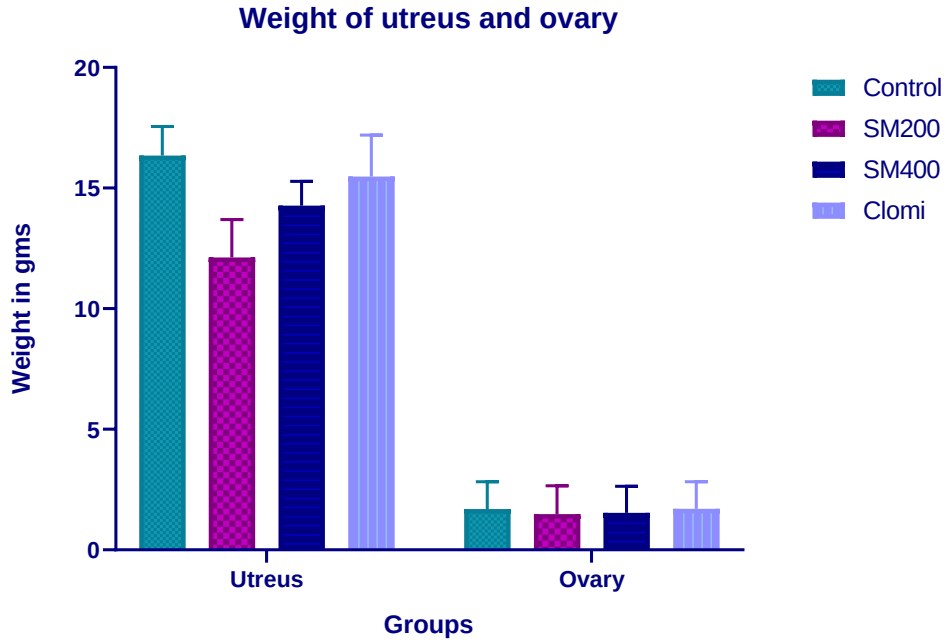
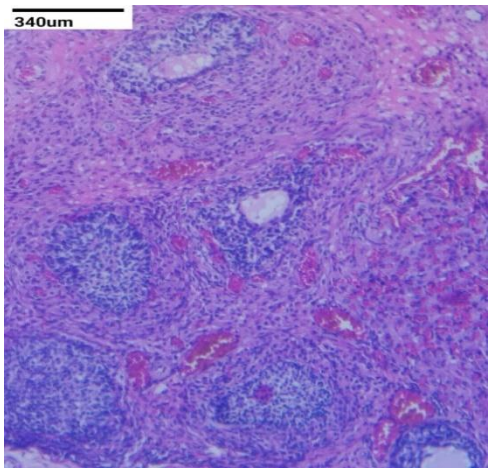


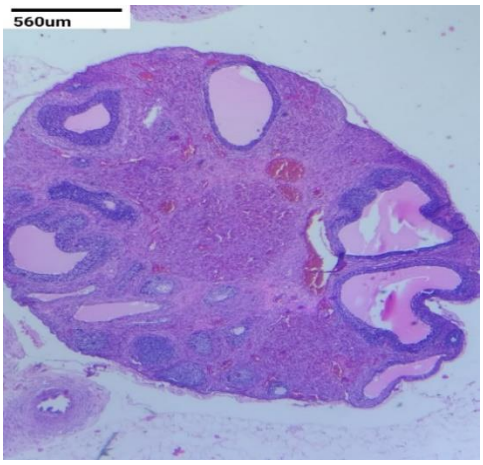
Fig.No.20. Histopathology slides of ovary

OVARY HISTOPATHOLOGY



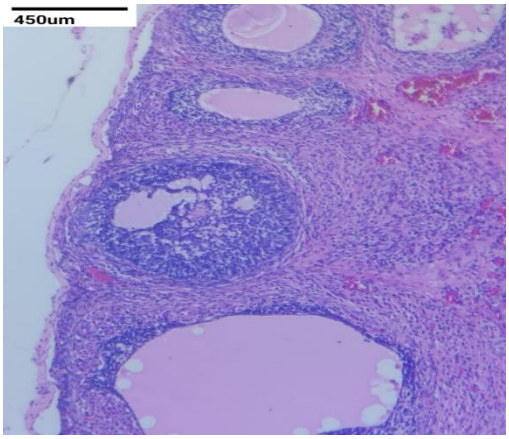
Normal

Normal control ovary with healthy follicles and corpus leutum.



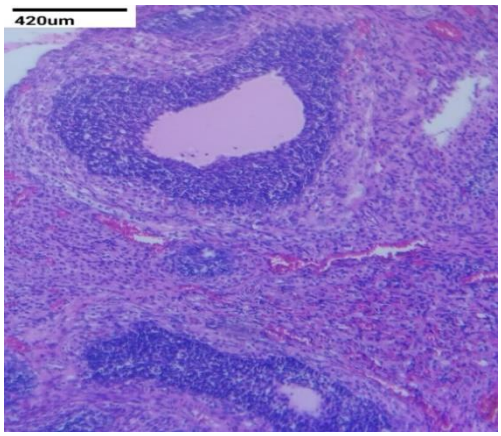
Induced

Induced group cystic follicles with thin granulosa layer, atretic follicles, stromal hyperplasia, and vacuolated stroma.



Low dose

200mg/kg showing healthy follicles in various stages of development and with congested blood vessel.



High dose

400mg/kg treated showing healthy follicle and healthy stroma.

Histopathological study of ovary tissue:

Histological studies of ovarian tissues of normal control group shows ovary with healthy follicles and corpus leutum and induced group shows cystic follicles with thin granulosa layer, atretic follicles, stromal hyperplasia, and vacuolated stroma.

Standard group and both doses of *Soodhaga Mezhugu* (*SM* –200mg and *SM*-400mg) showed some well-defined histological features with showing healthy follicles in various stages of development, congested blood vessel and healthy stroma.

Estimation of hormone level:

Hormonal Assay

The *Soodhaga Mezhugu* in the dose of 200mg and 400mg was administered orally for 10 days and the estimation of LH, FSH, and Progesterone, estradiol study was carried out. *SM* 400mg should significant increase in the hormone level estimated.

Serum levels of Progesterone and Estradiol were decreased in PCOS induced female Wistar rat. Decreased progesterone levels are also an indication of anovulation and *SM* successfully restores the level to normal. Decreased estradiol concentration due to inhibition of aromatase in PCOS induced group was significantly increased by administration of *SM* of (400mg/kg).

Administration of *SM* in the dose of 200mg/kg and 400mg/kg produced no significant effect on LH which was nearly similar to the normal and standard group. There was significant increase in the FSH level treated with *SM* 400mg/kg and the 200mg/kg was also increase in the level of FSH which was not statistically significant.

Progesterone level slightly significant compared to the normal and standard group. Estrogen level showed significant decreased after the administration of ***Soodhaga Mezhugu*** at the dose level of 200mg/kg when compared with standard and normal control group. Decreased estrogen level was insignificant. But in the dose level 200 mg/kg significantly increase the estrogen level.

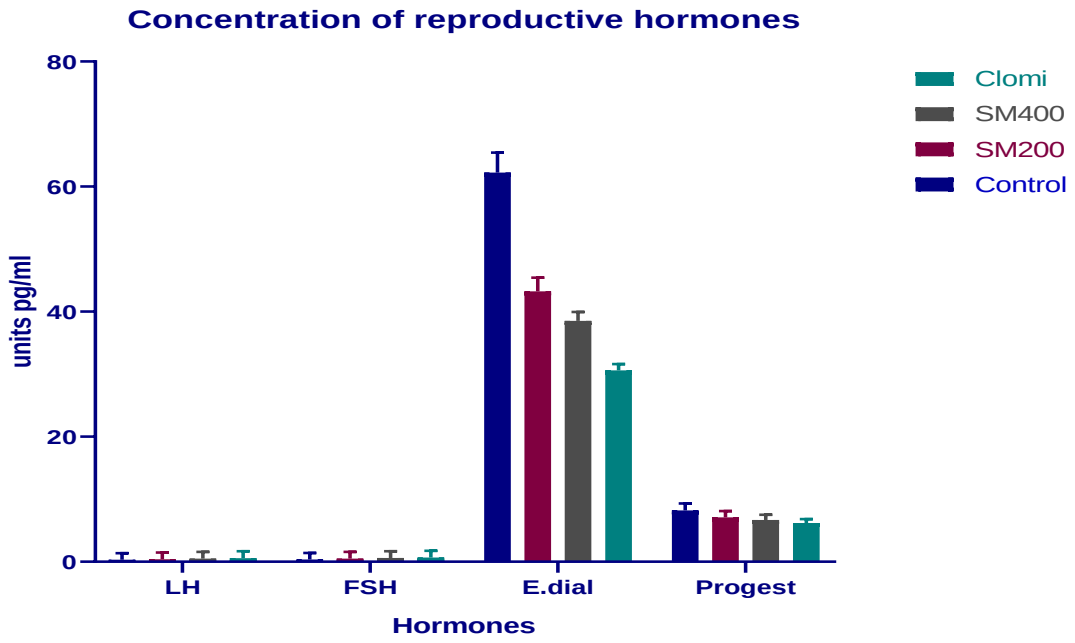
SM in the dose of 400mg/kg was increase the level of FSH and progesterone statistically significant, which is primary step for inducing ovulation.

Table No.30. Effect of *Soodhaga Mezhugu on* Serum Concentration of reproductive hormones of female Wister albino rat

S. No	Group	Treatment and dose	LH (IU/ml)	FSH (IU/ml)	Estrodial (pg/ml)	Progesterone (pg/ml)
1.	Normal	2ml/kg 2% CMC	0.32±0.05	0.36±0.04	62.24±3.2	8.2±1.12
2.	Test-I	SM200mg /kg	0.40±0.07	0.52±0.07	43.25± 2.2$^{**, a}$	7.1±1.00
3.	Test-II	SM400mg/kg	0.49±0.08	0.62± 0.07*	38.54± 1.4^{**,a}	6.7±0.82
4.	Standard	Clomiphene 10mg/kg	0.56±0.14	0.67± 0.10*	30.62± 1.0**	6.2±0.61

*N = 6. Values are expressed as Mean±SEM. *p<0.05;**p<0.01 V Normal control[a] p<0.01 Vs Standard.*

Chart. No.2. Effect of *Soodhaga Mezhugu on* Serum Concentration of reproductive hormones of female Wister albino rat



Antioxidant action in PCOS [119]:

- The abnormal oxidative stress in polycystic ovary syndrome (PCOS) could cause genetic instability and raise the risk of cancers. Antioxidants have positive effects on PCOS.
- The antioxidants involved in GSH recycling, regulation during the ovarian cycle and follicular maturation, oocyte maturation, ovulation, corpus luteum function, and steroidogenesis. The possible regulatory function of gonadotropins on antioxidants is also addressed here.
- Antioxidant closely related to female subfertility or infertility, oocyte maturation to fertilization and embryo development. Elucidation of the molecular mechanism underlying the involvement of antioxidants in follicular growth, ovarian cycle, oocyte maturation and ovulation is essential to creating possible protective effects of antioxidants.

Result:

Table.No.31. DPPH assay on *Soodhaga Mezhugu*

Concentrations (µg/ml)	**Absorbance**		**Percentage of inhibition**	
	Drug	**Standard**	**Drug**	**Standard (Ascorbic acid)**
12.5	1.3210	1.4044	9.50	21.90
25	0.8589	1.0782	27.01	40.04
50	0.5008	0.7121	43.39	60.40
100	0.1567	0.2921	55.82	83.75
200	0.1150	0.0692	67.58	96.15

**µg/ml: microgram per millilitre. Drug: SM (12.5-200µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)*

Chart.No.3. DPPH assay on *Soodhaga Mezhugu*

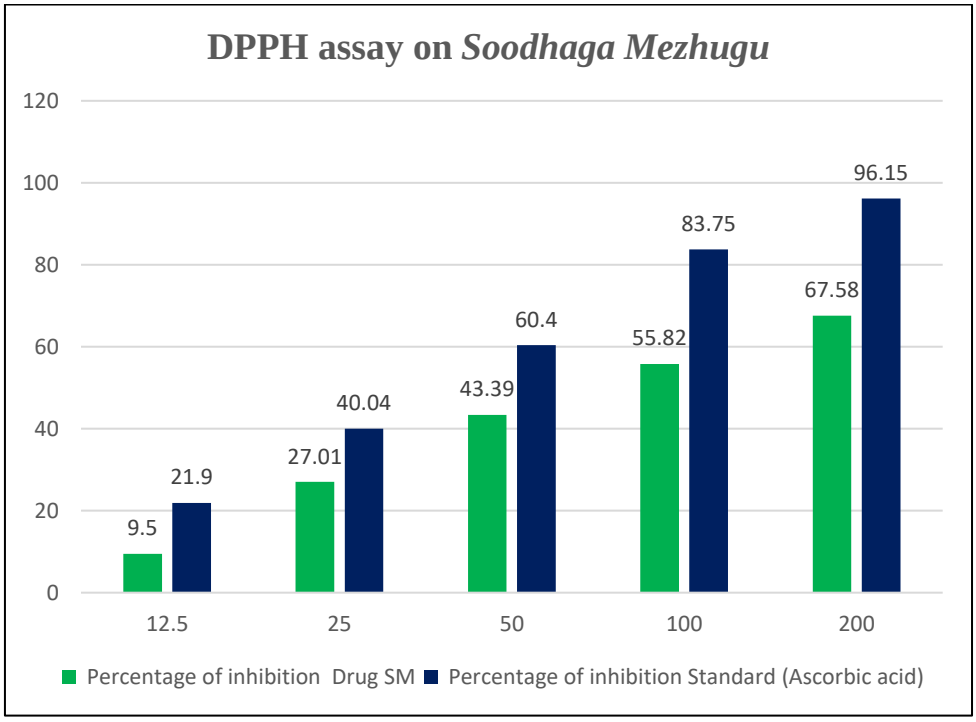
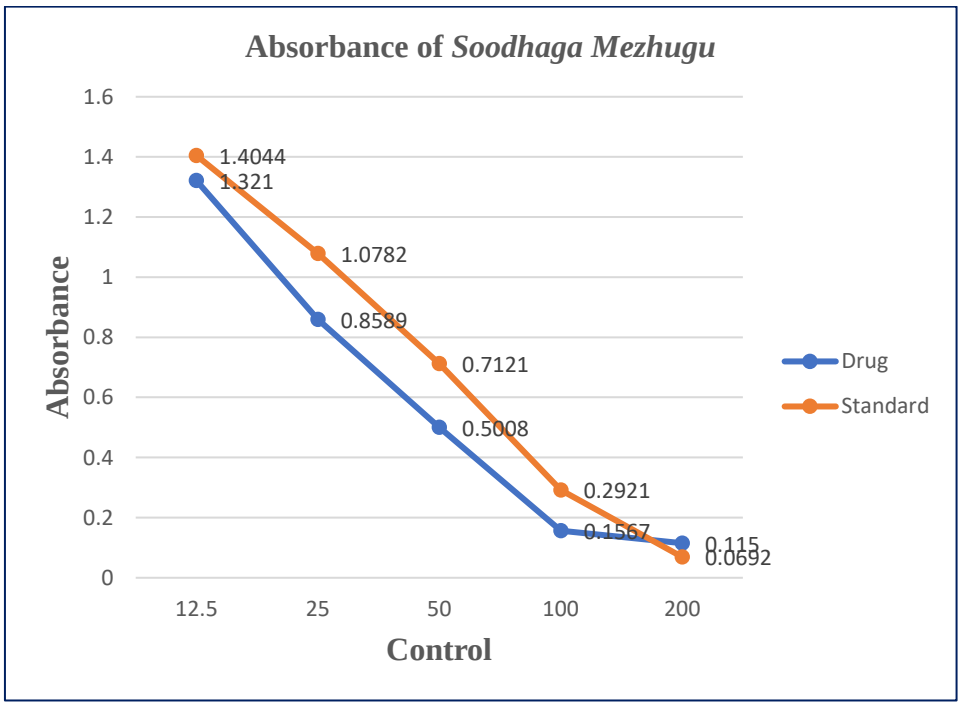


Chart no 4: Absorbance of *Soodhaga Mezhugu*



Discussion on Antioxidant activity in DPPH assay:

DPPH assay were used for the determination of anti-oxidant activity of the different extracts. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of SM extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2- picrylhydrazyl is formed and as a result of which the absorbance at 517nm of the solution is decreased.

In the present study, the SM extract was analyzed to decolorize DPPH and the free radical scavenging activity Ascorbic acid (10 mg/ ml DMSO) was used as a reference and result was expressed as the percentage decrease in absorbance. In the present study, the extract of *SM* was found to possess concentration dependent scavenging activity on DPPH radicals.

The values of DPPH free radical scavenging activity of the *SM* extract were given in (Table.No.31) expressed in the percentage. The extract of *SM* showed the highest DPPH scavenging activity (67.58%) at 200µg/ml and the lowest percentage of inhibition (9.50%) at 12.5µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (96.15%) at 200µg/ml and the lowest percentage of inhibition (21.90%) at 12.5µg/ml.

This indicated that % of inhibition increased with increase in concentration of both the standard and *SM* extract. The *SM* extract has less free radicle scavenging activity compared to the standard. From the present study, it was concluded that the *SM* extract has a marked low-level antioxidant activity at higher concentrations. Antioxidant capacities of the extracts were expressed in terms of IC_{50} value of the extracts and low IC_{50} value corresponds to a high antioxidant capacity.

Hence the in vitro study of DPPH assay free radicle scavenging activity shows a less antioxidant property when compared to standard drug ascorbic acid and IC_{50} value (79.9345µg/mL) of the extracts are high in level. Some other assays have to be studied to evaluate the efficacy of antioxidant activity in different assays and in vivo studies of *SM*. This assay confirms that the trial drug *SM* has significant antioxidant property.

6. CONCLUSION

Polycystic ovary syndrome (PCOS) is currently the leading cause of menstrual complications in women. There is lot of medications available but it has high cost effective and side effects, still need of safe drug with high effectiveness.

Keeping the aforesaid facts in mind there is a need to provide a solution, this study ***Soodhaga Mezhugu*** was chosen for PCOS (*Soodhaga vayu*) from the *Siddha* literature **"*Kosayee Anuboga Vaithiya Biramma Ragasiyum*"** written by ***Munusamy mudaliyar*** as a trial drug which was categorized by the department of AYUSH as a classical text.

The ingredients of the test drug are *Vengarathool (Sodium biborate), Valendhraholam (Commiphora myrrha), Kunguma poo (Crocus sativus)* and *Kirambu thylum (Syzygium aromaticum).* The drugs were identified and authenticated by the experts of Gunapadam (Pharmacology), Government Siddha Medical College, Chennai-106.

The drugs were purified and processed as per *Siddha* literature. The fine particles in it enhance the curative potential with shelf life of Five years.

Organoleptic characters of the *SM* show GMP based on the *Siddha* parameters. Throughout the study standardization, safety and efficacy were tested thoroughly in the following procedures such as physiochemical, phytochemical, biochemical, HPTLC, instrument analysis (FTIR, ICPOES, SEM, XRD) Toxicity studies, Pharmacological studies.

In physicochemical analysis *SM* contain specific gravity, acid soluble ash with high total ash value (42.71%) ensure the purity and safety of the drug. Water soluble ash value is 37.67% which is a part of total ash value has diffusion capacity of the drug. Highly alkaline pH value shows good absorption in alkaline medium (Intestine). Loss on drying gives the total of volatile content and moisture present in the drug. The value of LOD is 11.32% this ensures maximum stability and better shelf life. The alcoholic and water-soluble extracts show that alkaloids are more soluble in water than alcohol.

Phytochemical analysis revealed the presence of glycosides, saponin, proteins, amino acids, diterpenes, gum and mucilage in the drug and they are responsible for the ovulation inducing activity.

Moreover, the Bio-chemical analysis exhibited the presence of potassium, zinc, aluminium, iron and sulphate in the drug which might be responsible for ovulation inducing activity and regulating hormone system. The presence of these minerals may play an important role in the functioning of various enzymes in biological systems and have immunomodulatory functions.

Soodhaga mezhugu is rich in various Phyto-components that were analysed by several qualitative and quantitative analysis. Further, confirmation for the presence of Phytosterols like sterols, β-sitosterol etc along with steroid derivatives has been performed by HPTLC.

The anti-microbial and anti-fungal activity shown effective control that at low and medium doses against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Aspergillus niger*.

FTIR shows functional groups such as Alcohol, Phenols, 1^0 Amines, Alkanes, Alcohols, carboxylic acids, esters and ethers, Alkyl halides, Aliphatic amines, Alkyl halides, Alkyl halides. Phenolic group of compounds exert antioxidant activity. Alcohol, Amines, Alkanes, Carboxylic acid and Alkyl halides which enhance and regulate the hormones, have ovulation activity.

SEM identifies the microparticles in the trail drug. Presence of microparticles (5-2 Microns) in medicine increases the drug absorption. Detection of heavy metals in the drug through ICP-OES like lead, cadmium, mercury arsenic presents the below detective level ensure the safety of the trial drug *SM* and the presence of Ca, Fe, Mg, Na & P. Results of XRD shows crystalline nature of the *SM*.

In acute toxicity study single oral dose of the *SM* showed no mortality of rats even under higher dosage levels indicating the high margin of safety of this extract. In Repeated 28 days oral toxicity studies results ensure the herbo-mineral formulation of *SM* can be considered to be safe drug for prolonged duration use as revealed by toxicological studies.

Pharmacological studies had shown that the trial drug increased folliculogenesis at the dose level of 400mg/kg and this marked effect was ensured with the histological evaluation of uterus of experimental rats also.

The hormonal assay of the female Wister albino rats was evaluated using the estimation of hormones like FSH, LH, Estradiol, Progesterone. Significant increase in the hormonal levels of FSH, Progesterone, decrease the LH, estradiol level was recorded statistically 400mg/kg of the trail drug. The increase in the level of hormones proved to be the prime reason for ovulogenic activity.

To conclude, the drug *Soodhaga Mezhugu* beneficiary values for the therapeutic efficacy for ovulation, it can be concluded that *Soodhaga Mezhugu* could be a scientifically validated and had shown its sphere of action over the female reproductive system especially over the ovaries in the condition called PCOS.

7. SUMMARY

In this present study the trial drug herbo mineral formulation of *Soodhaga Mezhugu* was taken as the compound drug preparation for Polycystic Ovarian Syndrome were taken from the Siddha classical literature ***"Kosayee Anuboga Vaithiya Biramma Ragasiyum"*** written by ***Munusamy mudaliyar.***

- Selection of the trial drug was approved by the screening committee.
- Introduction of this thesis elucidate about the women's lifestyle disorders, *Soodhagavayu* compared to the modern aspect PCOS, explain about the signs and symptoms, modern treatments.
- Review of literature explain the reference of the trial drug, *Gunapadam* and modern aspect of the drug, disease, treatment and also describe the animal model for ovulation inducing activity.
- Systematic identification of trail drug through microscopic and macroscopic method and authenticated by *Gunapadam* department experts. All the ingredients in the preparation detoxified and SOP was done at *Gunapadam* Lab.
- Drug standardization was done through the physicochemical property, acid and basic radicles, the functional group and nanoparticles were identified by instrumental analysis.
- Toxicological study was made according to OECD guidelines comprising both acute and repeated oral dose 28 days toxicity studies in wistar albino rats. It showed the safety of the drug which attributes its utility in long time administration.
- Pharmacological studies were completed. It discovered the Ovulation inducing activity, Estimation of hormone level and Antioxidant activities of *Soodhaga Mezhugu* Results and discussion gives the essential validations to prove the potency of the drug.
- Conclusion gives a hoarded form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

8. FUTURE SCOPE

Soodhaga Mezhugu indicated for *Soodhaga Vayu* as per *Siddha* literature is being proved that the drug its role in the treatment of PCOS.

With reference to the in *vivo* studies in Female Wistar Albino rat, clinical trials should be conducted in future to authenticate the traditional use of *SM* in menstrual irregularities and fertility issues in humans.

SEM depicted the presence of Nano particles, the active compound exhibiting the therapeutic efficacy should be analysed. The composition of the compound in micro size and their percentage of presence in *SM* should be elucidated in future by Dynamic Light Screening and Nuclear Tracking Analysis.

More recently, with the advance of gene technology many new animal models that can aid in illumination of PCOS pathophysiology have been established.

This is first study where implication of ***Soodhaga Mezhugu*** has shown the potential to manage PCOS, which could be considered as a novel component for future drug development and should be screened for rest of the pharmacological activities like Ovulogenic activity, Hormonal assay, Anti-oxidant activity to rule the effective pathways so that, the exact benefit and aim of the study would be fulfilled.

BIBLIOGRAPHY

1. Thirukkural, Thiruvalluvar, Periyannan urai, Arathupaal, No:56, Thirukkural Athikaaram, pg.no:12.
2. https://images.app.goo.gl/5Zf6TzDQZYLgkYk48.
3. Nadir Anvarbhai Dawoodani, Chairman of Agakhan Health Service India, Jhunjhunu, Rajasthan, India, Journal of Mass Communication & Journalism, 2013, 3:4 Dol: 10.4172/2165-7912-1000157.
4. NLM (2015), "Women's Health", Medical Subject Headings(MSH) Retrieved 6 July 2016.
5. WHO 2016, Women's Health.
6. Gronowski, AnnM; Schindler, Emily 1. (2014). "Women's Health" Scand Jclin Lab InvestSupp.244:2- doi:10.309/00365513.2014.936672.PMID25083885.,in Bergmeyer (2014).
7. Gilden RC, Huffling K, Sattler B. Pesticides and health risks. Journal of Obstetric, Gynecologic & Neonatal Nursing. 2010 Jan 1;39(1):103-10.
8. VG Padubidri Shirish N Daftary, Howkins & Bourne Shaw's Textbook of Gynaecology 13th Edition, Polycystic Ovarian Syndrome (PCOS) or Disease (PCOD), 2004, pg.no: 353.
9. Norman RJ, Dewailly D, Logro Rs, Hickey TE, Polycystic Ovary Syndrome. Lancet.2007:37:685-697. [Pub Med].
10. Rzeszowska M, Kotarski J, Polak G (2016). "Anti-Mullerian hormone: Structure, properties and appliance". Grinekdogia Polska. 87(9): 669-674. Doi:10.5603/gp.2016.0064. PMID27723676.
11. Stephanie Watson, Medically reviewed by Debra Sullivan, PhD, MSN, RN, CNE, COL, Polycystic Ovary Syndrome (PCOS): Symptoms, Causes, and Treatment, November 1, 2018.
12. Nidhi R, Padmalatha V, Nagarathna R, Amritanshu R. Prevalence of polycystic ovarian syndrome in Indian adolescents. Journal of pediatric and adolescent gynecology. 2011 Aug 1;24(4):223-7.
13. Stephanie Watson. Healthline. Polycystic Ovary Syndrome (PCOS): Symptoms, Causes, and Treatment. September 27, 2017 available at: https://www.healthline.com/health/polycystic-ovary-disease#pregnancy-and-pcos.

14. Yen SS. Regulation of the hypothalamic–pituitary–ovarian axis in women. Reproduction. 1977 Sep 1;51(1):181-91.
15. PCOS awareness Association: ovarian hyperstimulation syndrome PCOS treatment complication. 2017. available from http://www.pcosaa.org/ohss.
16. Richard Scott Lucid, MD., PACOG, Polycystic Ovary Syndrome Treatment & Management, Feb 28, 2018.
17. Pasquali R, Gambineri A, Therapy in endocrine disease, Treatment of Hirsutism in the Polycystic Ovary Syndrome, Eur J Endocrinal 2013; 170: R75-R90. [PubMed].
18. PCOS awareness Association: ovarian hyperstimulation syndrome PCOS treatment complication. 2017. available from http://www.pcosaa.org/ohss.
19. Dr.R.Thiyagarajan.LIM., Gunapadam Thathu Vaguppu, Dept. of Indian medicine & Homoeopathy, Edition 2009, Page no: 269-273.
20. K.S.Murukaesa Mudhaliyar, Siddha Materia Medica (medicinal plants division), published by Indian Medicine and Homeopathy Dept, Chennai-600106, and Page no :a) 808, b) 350, c) 111.
21. Turkez H., Geyikoglu F., Tatar A., Keles M.S., Kaplan I.The effects of some boron compounds against heavy metal toxicity in human blood. Exp Toxicol Pathol. 2012 Jan; 64(1-2):93-101. Epub 2010 Jul 20. Article.
22. Properties of Borax | Hunker, Available at https://www.hunker.com > Home Hacks & Answers > Housekeeping > House Cleaning-Nov 20, 2010.
23. W.H .Hul , Physiological Material Medica.
24. Nadkarni K.M, Indian Materia Medica, Published by Prakashan Pvt Ltd, Bombay, Vol II, 1976, pg.no A. 357-360.
25. Commiphora myrrha -Germplasm Resources information Network (GRIN), Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Retrieved 2009-01-15.
26. Indian Medicinal Plants a Compendium of 500 Species, Vol-2, 13 Jan 2000, Pg.no: 173.
27. Amal A. Mohamed, Chemical composition of essential oil and in vitro antioxidant and antimicrobial activities of crude extracts of Commiphora myrrha resin, industrial crops and products volume 57, Pg.no: 10-16.
28. K.S.Mhaskar, E.Blatter et al, Indian medicinal plants, vol 10, published by orient torgman limited, reprinted 1997 28a & 28b Pg.no: 3402-3403.

29. Neeraj Tandon Co.od & et, Quality Standards of Indian Medicinal Plants, Vol-9, Medicinal Plants Unit, ICMR, New Delhi, 2011, Pg.no:92-99.
30. The Wealth of India, Publications Information directorate, CSIR, New Delhi, Vol XI, Reprinted 1988.
31. Compendium of Indian medicinal plants, Edition-II, Vol- III, pg.no 231.
32. Neeraj tendan, Paul sharma, Quality of Indian Medicinal Plants, Volume 14, Medicinal Plants Division, Indian Council of Medical research, New Delhi, 2017, Pg.no: 314-317.
33. Ramachandran, Thirumoolar karukkadai Vayithiyam 600, Published by Rathna Nayaker and Sons, Chennai79, Thirumagalvilasa press, P. no 19.
34. Kandhasamy MudhaliyarV, Aaviyallikum Amudhamurai Churukkam, Published by Siddha Maruthuva Nool Veliyitu Pirivu, Indian Medicine and Homeopathy dept., Chennai 106, part 1, 3rdedition, 2010;71.
35. Thiyarajan,Agasthiyar Aayul Vedham-1200, Published by Thamarai Noolagam, Chennai 26, first edition Oct 1999, P.no 112.
36. Murugesa Mudhaliyar, Yugi Vaidiya Chinthamani, published by Dept. of Indian Medicine and Homeopathy Chennai 106, Second Edition 2005, P. no 52.
37. Ramachandran S P, Agasthiyar Amudhakalai Gnanam, Published by Thamarai Noolagam, Chennai 26,1996 may; Pg no 229.
38. Ramachandran S P,Pathinen Siddhargal Paadiya Vaidiya Sillaraikovai,Part 1, Published by Thamarai noolagam,Chennai 26, April;1996 P. no 2.
39. Kalathoor Kandhaswamy Mudhaliyar, Pathinen Siddhargal Naadi Saasthiram, Published by Rathna Nayaker and Sons, Chennai-79, 2012; P. no 42.
40. Guilherme Barbosa. Polycystic Ovary Syndrome (PCOS) and Fertility: Division of Endocrinology, IPEMED Medical School. published 21 January 2016:P.no 58-65.
41. Ahmed Kabel M. Polycystic Ovarian Syndrome: Insights into Pathogenesis, Diagnosis, Prognosis, Pharmacological and Non-Pharmacological Treatment. Journal of Pharmacological Reports. 2016.
42. Polycystic Ovarian Syndrome definition available at: https://www.nature.com/articles/nrendo.
43. Nithi R, Padmalatha V, Nagarathna R, Amirtanshu R, Prevalence of polycystic ovarian syndrome in Indian adolescents. Journal of pediatric and adolescent gynecology. 2011 Aug 1;24(4):223-7.

44. Azziz R. Diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. The Journal of Clinical Endocrinology & Metabolism. 2006 Mar 1;91(3):781-5.
45. AntonioSimoneLaganà. Metabolism and Ovarian Function in PCOS Women: A Therapeutic Approach with Inositols. International Journal of Endocrinology. Hindawi Publishing Corporation: Volume 2016.
46. Pelusi 'B', Gambineri A, Pasquals R, Type 2 diabetes and the Polycystic Ovary Syndrome, Minerva Ginecol-2004. Feb; 56(1):41-51.
47. Ahmed Kabel M. Polycystic Ovarian Syndrome: Insights into Pathogenesis, Diagnosis, Prognosis, Pharmacological and Non-Pharmacological Treatment. Journal of Pharmacological Reports. 2016.
48. Guilherme Barbosa1. Et al., Polycystic Ovary Syndrome (PCOS) and Fertility. Open Journal of Endocrine and Metabolic Diseases. 21 January 2016. Available : http://www.scirp.org/journal/ojemdhttp://dx.doi.org/10.4236/ojemd.2016.
49. Symptoms of PCOS available at: https://bestpractice.bmj.com/topics/en-us/141
50. Ahmed Kabel M. Polycystic Ovarian Syndrome: Insights into Pathogenesis, Diagnosis, Prognosis, Pharmacological and Non-Pharmacological Treatment. Journal of Pharmacological Reports. 2016.
51. Ahmed Kabel M. Polycystic Ovarian Syndrome: Insights into Pathogenesis, Diagnosis, Prognosis, Pharmacological and Non-Pharmacological Treatment. Journal of Pharmacological Reports. 2016.(50)
52. Ahmed Kabel M. Polycystic Ovarian Syndrome: Insights into Pathogenesis, Diagnosis, Prognosis, Pharmacological and Non-Pharmacological Treatment. Journal of Pharmacological Reports. 2016. (51)
53. BMJ best practice: Jan 2018. Complication available at: https://bestpractice.bmj.com/topics/en-us/141) (52)
54. Sylwia Bednarska A. et al.,The pathogenesis and treatment of polycystic ovary syndrome: Department of Clinical Endocrinology Advances in Clinical and Experimental Medicine. 2017;26(2): P. no359–367.
55. Oliver Oakley. et al., Laproscopic surger Animal Models For the Study Of Polycystic Ovarian Syndrome. 2001.
56. Stuart Campbell. The advantage and disadvantage of IVF, Create fertility. Available at: https://www.createfertility.co.uk/blog/the-advantages-and-disadvantages-of-ivf.

57. Corey Whelan. Healthline. Can My Diet Relieve Symptoms of Polycystic Ovary Syndrome (PCOS) [August10,2016] available at: https://www.healthline.com/health/pcos-diet
58. Siddha treatment guidelines for selected diseases published by National Institute of Siddha, Ministry Of Polycystic Ovarian Syndrome, Endocrino Metab 23(3):193-202, September 2011 available at: http:// www.enm-kes.org.
59. Yoga for Polycystic Ovarian Syndrome. Alternative and Complementary Therapies19(2):101-106. [April 2013] Available from: https://www.researchgate.net/publication/305775488_Yoga_for_Polycystic_Ovarian_Syndrome. (58)
60. Oliver Oakley. et al., Animal Models for the Study of Polycystic Ovarian Syndrome.
61. Dr. R. Thiyagarajan, LIM., in Tamil., Dr. Anaivaari R. Anandan, PH.D., Dr. M. Thulasimani, M.D. (PHARM)., Siddha Materia Medica (Mineral & Animal Kingdom), Dept. of Indian Medicine & Homoeopathy, Chennai – 106, First edition, 2008, Pg.no:62.
62. Sarkar PK, Prajapati PK, Shukla VJ, Ravishankar B. Evaluation of processed borax as antidote for aconite poisoning. Journal of ethnopharmacology. 2017 Jun 9;205:138-46.
63. Maver T, Maver U, Stana Kleinschek K, Smrke DM, Kreft S. A review of herbal medicines in wound healing. International journal of dermatology. 2015 Jul;54(7):740-51.
64. Yaribeygi H, Zare V, Butler AE, Barreto GE, Sahebkar A. Antidiabetic potential of saffron and its active constituents. Journal of cellular physiology. 2019 Jun;234(6):8610-7.
65. Karimi E, Oskoueian E, Hendra R, Jaafar HZ. Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules. 2010;15(9):6244-56.
66. Hashimova UF, Kamilova NK, Babaev KF, Shukurova PA, Hasanova SI, Abbasov RY. Effects of saffron (Crocus sativus l. iridaceae) on bloodlevel of follicle-stimulating hormone, and number and dynamics of body weight of offspring in female rats. Advances in gerontology= Uspekhi gerontologii. 2017;30(3):436-41.

67. http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler/syzygium-aromaticum.html.
68. Radünz M, da Trindade ML, Camargo TM, Radünz AL, Borges CD, Gandra EA, Helbig E. Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (Syzygium aromaticum, L.) essential oil. Food chemistry. 2019 Mar 15; 276:180-6.
69. El-Maati MF, Mahgoub SA, Labib SM, Al-Gaby AM, Ramadan MF. Phenolic extracts of clove (Syzygium aromaticum) with novel antioxidant and antibacterial activities. European Journal of Integrative Medicine. 2016 Aug 1;8(4):494-504.
70. S.B.Ramachandran, *Kosayee Anuboga Vaithiya Biramma Ragasiyum,* Part-2, March 1999, Page no-227.
71. Thiyagarajan.R. GunapadamThathu seeva vaguppu.Published by Department of Indian Medicine and Homoeopathy., Reprinted 2004; Page no-437.
72. Sarakku-Suthi Muraigal, Published by Siddha Maruthuva Nool Veliyitu Pirivu, Indian Medicine and Homoeopathy dept., First edition, 2008 pg.no: 4.
73. Lohar DR. Protocol for testing: Ayurvedic, Siddha and Unani Medicines. Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad.
74. WHO guidelines
75. Prashant Tiwari et al. Phytochemical Screening and Extraction a Review, IPS, Jan-March 2011 Volume 1.
76. Anonymous, 1998, Bio chemical Standards of Unani formulations, Part3, CCRUM, New Delhi, P.no.58-60.
77. Anonymous, 1998, Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, 25-28, p.no 61-73.
78. National Committee for Clinical Laboratory Standards. (1993a). Performance Standards for Antimicrobial Disk Susceptibility Tests-Fifth Edition: Approved Standards M2-A5. NCCLS, Villanova, PA.
79. Fourier Transform Infra-red Spectroscopy available at: https://www.lpdlabservices.co.uk/analytical_techniques/chemical_analysis/ftir php.
80. http://www.hitachihightech.com/global/product/science/tech/ana/icp/descripts/icp-oes.html/
81. http://www-odp.tamu.edu/publication/tnotes/tn29/technot4.htm.

82. Scanning Electron Microscope available at: https://serc.carleton.edu/ research_education/ geochemsheets/techniques/SEM. html.
83. X-ray powder diffraction available at:https://serc.carleton.edu/research education/geochemsheets/techniques/XRD.html.
84. Organization for Economic Cooperation Development (OECD) Guideline, 423, 2000. Guideline Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24.
85. Schlede E., Mischke U., Diener W. and Kayser D 1992;66: 455-470.
86. OECD Guidelines for the Testing of Chemicals (No. 407, Section 4: Health Effects) "Repeated Dose 28-Day Oral Toxicity in Rodents" (Adopted on 12 May 198 1 and Updated on 27 July 1995.)
87. Asolkar L.V, Kakkar K.K, Chakre O.J, Glossary of Indian medicinal plants with active principles, Publications & information directorate council of scientific & Industrial research New Delhi.
88. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acasia confuse* bark and heartwood. J Agric Food Chem. 2001;49:3420-3424.
89. Chang et al., DPPH assay: 2001.
90. Thiyagarajan.R. GunapadamThathu seeva vaguppu. Published by Department of Indian Medicine and Homoeopathy., Reprinted 2004; P.No.269,302.
91. Boron Uses – Boost Bone Density and Much More, By Jillian Levy, CHHC, October 25, 2018.
92. https://organixx.com - 4 ways that Myrrh supports a healthy body.
93. Hashimova UF, Kamilova NK, Babaev KF, Shukurova PA, Hasanova SI, Abbasov RY. Effects of saffron (Crocus sativus I. Iridaceae) on blood level of follicle-stimulating hormone and number and dynamics of body weight of offspring in female rats. Advances in gerotology = Uspekhi gerontologii, 2017; 30(3):436-41.
94. EI-Maati MF, Mahgoub SA, Labib SM, Al-Gaby AM, Ramadan MF. Phenolic extracts of clove (Syzygium aromaticum) with novel antioxidant and antibacterial activities. European Journal of Integrative Medicine. 2016 Aug 1;8(4):494-504.
95. Ishihara K,Yamanami K, Takano M.Zinc bioavailability is improved by the microniced dispersion of zinc oxide with the addition of L-histidine in zinc-

deficient rats. Journal of Nutritional Science and Vitaminology, 2008; 54(1): 54-60.

96. Ketan T.et all., NCBI.Drug Solubility: Importance and Enhancement Techniques[2012jul5];availabat:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3399483/
97. drsheiland.com/what-is-alkaline-and-why-is-it-important.
98. The Ash Content Of A Crude Drug Biology Essay Published [23rd March, 2015]; available at : https://www.ukessays.com/essays/biology/the-ash-content-of-a-crude-drug-biology-essay.php.
99. http://www.slideshare.net/mobile/sudhaeukandibanda/glycosides-64277083.
100. Brinda et al., (1981); Siddiqui and Ali (1997) and Savithramma et al, (2011)
101. http://www.globalhealingcentre.com/natural-health/what-are-saponin.
102. www.cyperlipid.org>simple>simp00043.
103. https://www.researchgate.net/
104. Elson M. Haas, M.D., Role of Potassium in Maintaining Health 17 Jul 2011. http://hkpp.org/patients/potassium-health.
105. Organic facts.17 Surprising Benefits Of Iron, organic facts. Available at:http:// at:https://www.organicfacts.net/health-benefits/minerals/health-benefits-of-iron.html.
106. Laboratories de Biochimie des interactions Micronutrients, University J.Fourier, Facult de pharmacy, Tronche, France, The role of zinc in reproduction. Hormonal mechanisms, 1992 Jan-Mar, 32 363-82.
107. Magdalena Wszelaki, Using the mighty zinc to balance your hormones, Mar 27, 2019.
108. htts://www.hydro.com/en/about-aluminium/aluminium-and-health/.
109. DC Nutrition. Nutritional & Supplemental Information. Available https://www.dcnutrition.com/nutritional-supplemental-information/.
110. Huan-xia.Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. [September–December 2014]; Pages 183-190.
111. Properties and Uses of Amines. [January 15, 2016]: available at: https://byjus.com/chemistry/uses-of-amines/
112. Sudhakar. Regulation of Estrous cycle using Combination of Gymnema sylvestre and Pergularia daemia in Estradiol Valerate induced PCOS rats.

113. Functional and application of carboxylic acid. Available at: https://www.ck12.org/book/From-Vitamins-to-Baked-Goods:-Real-Applications-of-Organic-Chemistry/section/6.3/
114. Lucas Karl Hahn What are the advantages of nanomedicine or nanoparticles in medicine. [Mar 15, 2015]; Available at:https://www.quora.com/What-are-the-advantages-of-nanomedicine-or-nanoparticles-in-medicine.
115. Harvey H et.al.Stimulation of gonodotropic hormones with mineral mixtures containing amino acids chelates, Sep 27, 1988.
116. Alisa. et all., The benefits of magnesium for hormonal balance [september 19, 2016]: available at https://www.floliving.com/benefits-of-magnesium-for-hormonal-balance/.
117. Bell S. Overview of Anti-oxidants: Emphasis on Raisins. Int J Nutr Wellness. 2009:10.
118. Khaoulani, M. Kassem,The Agl-HgS-AS2S3,glassy System: Macroscopic Properties and Raman scattering studies, Journal of Alloys and Compounds, Vol 685,Page: 752-760.
119. Role of Oxidative Stress in Polycystic Ovary Syndrome Article in Current Women.Availableat:https://www.researchgate.net/publication/228470687.

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy

An ISO 9001:2008 approved institution
Old Mahabalipuram Road,
Thoraipakkam, Chennai - 600 097.

ISO 9001 : 2008
Reg. No : RQ91/3038

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

L. Uday Metha
Secretary & Correspondent

Dr. Grace Rathnam, M.Pharm, Ph.D
Principal

APPROVAL CERTIFICATE

This is to certify that the project titled "SCIENTIFIC VALIDATION OF "***SOODHAGA MEZHUGU***" FOR OVULATION INDUCING ACTIVITY, ESTIMATION OF HORMONE LEVEL AND ANTIOXIDANT ACTIVIT IN female wistar albino rats" has been approved by the 53rd IAEC.

IAEC no: 01/321/PO/Re/S/01/CPCSEA dated 12/10/2018

C.L. Baid Metha College of Pharmacy CHENNAI-97 Thoraipakkam

P. Muralidharan

Dr.P.Muralidharan

(Member Secretary)

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....L. KAVINILAVU.....

For participating as ~~Resource Person~~ / Delegate in the Twenty Fourth Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 24th to 28th April 2017.

Dr.N.KABILAN, M.D.(S),Ph.D.,
PROF & HEAD DEPT.OF SIDDHA

Prof.**Dr.T.BALASUBRAMANIAN,** M.D.,D.L.O.,
REGISTRAR

Prof.**Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

NATIONAL SEMINAR ON

"RESEARCH METHODOLOGY AND PUBLIC HEALTH INITIATIVE THROUGH SIDDHA SYSTEM OF MEDICINE"

(RM & PHISSM – 2018)

6TH & 7TH APRIL 2018

प्रमाण पत्र
CERTIFICATE

सिद्ध क्षेत्रीय अनुसन्धान संस्थान
पूजप्पुरा, तिरुवनंतपुरम, केरल
SIDDHA REGIONAL RESEARCH INSTITUTE
Poojappura, Thiruvananthapuram, Kerala

केन्द्रीय सिद्ध अनुसन्धान परिषद्
(आयुष मंत्रालय, भारत सरकार)
CENTRAL COUNCIL FOR RESEARCH IN SIDDHA
Ministry of AYUSH, Govt. of India

This is to certify that Dr./Shri/Smt. Kavinilavu L, GSMC, Chennai has ~~participated~~/presented a paper entitled A method of Traditional Housing in Ancient Times in the National Seminar on "Research Methodology and Public Health Initiative through Siddha System of Medicine" (RM & PHISSM – 2018) organized by Siddha Regional Research Institute, Thiruvananthapuram on 6th & 7th April 2018 at Dr. M R DAS Convention Centre, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala.

डॉ. ए. कनगराजन / Dr. A. Kanagarajan
Organizing Secretary and Convenor

प्रो.डॉ.आर.एस. रामस्वामी / Prof. Dr. R. S. Ramaswamy
Director General, CCRS

CLINICAL RESEARCH & HOSPITALS (P) LTD

National Conference on

HERBAL MEDICINE AND ETHNOPHARMACOLOGY

Date: 06.04.2017; Venue: TICEL Biopark.

This is to certify that Ms./Mr./Dr. L. KAVINILAVU *from* DEPT. OF PHARMACOLOGY, GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI *attended the National Conference on "**Herbal Medicine and Ethnopharmacology**" conducted in V.S. Clinical Research & Hospitals (P) Ltd., Chennai, Tamil Nadu. He/She presented a paper/poster in the topic* ~~STA~~ EFFECT OF CARDIO PROTECTIVE POTENTIALS OF SIDDHA .....

T. Mathangi

Dr. T. Mathangi
Scientist & Coordinator

Dr. L. Lokoranjan
Chairman & Managing Director

GOVERNMENT SIDDHA MEDICAL COLLEGE

Arumbakkam, Chennai, 600106

This certificate is awarded to Dr./Mr./Ms. .....L..KAVINILAVU.....

for participating as a resource person / delegate in the seminar on

"Orientation to research Methods"

Organised by Suzhumunai Scientific forum Government Siddha Medical College on 22 March 2018

Dr. P. Manickam
Scientist E
(ICMR) National Institute of Epidemiology

Dr. K. Kanakavalli
Principal
Govt. Siddha Medical College

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

CHENNAI – 600 119

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH

(CPCSEA Approved)

WORKSHOP ON

TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS

CERTIFICATE

This is to certify that Dr./~~Mr.~~/~~Ms.~~ L. KAVINILAVU

of Govt. Siddha Medical College, Chennai has participated in the two-day workshop on "TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai during 31st January – 1st February 2018.

B. Sheela Rani

Chair Person & Coordinator
Dr. B. SHEELA RANI
Director (Research)

Convener
Dr. R. SELVARAJ
Scientist In-charge

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....S. SABARMATHI.....

For participating as ~~Resource Person~~ / Delegate in the Twenty Fourth Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 24th to 28th April 2017.

Dr.N.KABILAN, M.D.(S),Ph.D.,
PROF & HEAD DEPT.OF SIDDHA

Prof.**Dr.T.BALASUBRAMANIAN,** M.D.,D.L.O.,
REGISTRAR

Prof.**Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

Government Siddha Medical College

Arumbakkam, Chennai – 600 106

CERTIFICATE

Certified that the samples submitted for identification by Dr.L.Kavinilavu PG Scholar, Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

Ingredients of *Soodhaga Mezhugu:*

1. *Vengarathool [Sodium biborate]*
2. *Valendhrapolam [Commiphora myrrha]*
3. *Kunguma poo [Crocus sativus]*
4. *Kirambu thylum [Syzygium aromaticum]*

Date: 12.3.18

Place: Chennai

M.Saravanadevi 12/3/18

PG Department of Gunapadam

Dr. M.D. SARAVANA DEVI M.D. (S)
PROFESSOR
H.O.D. Department of Gunapadam
Govt. Siddha Medical College,
Chennai - 600 106.